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## Hybrids and Genetic Interactions of Mole Salamanders (*Ambystoma jeffersonianum* and *A. laterale*) (Amphibia: Caudata) in New York and New England

JAMES P. BOGART<sup>1</sup> AND MICHAEL W. KLEMENS<sup>2</sup>

### CONTENTS

Abstract .....	2
Introduction .....	2
Acknowledgments .....	3
Historical Overview .....	3
Reproductive Mechanisms .....	7
Nomenclature .....	8
New York and New England <i>Ambystoma</i> .....	9
Materials and Methods .....	10
Collection .....	10
Electrophoresis .....	10
Blood Cell Size .....	11
Chromosomes .....	12
Site Designations .....	12
Genotype Analyses .....	13
Results .....	14
Chromosomes .....	15

<sup>1</sup> Professor, Department of Zoology, University of Guelph, Guelph, Ontario, Canada.

<sup>2</sup> Research Associate, Department of Herpetology, American Museum of Natural History; Director, Program Development, Wildlife Conservation Society.

Area of Erythrocytes .....	16
Electrophoresis .....	17
Diagnostic Alleles .....	17
Rare Alleles .....	20
Homozygosity and Reversals in Hybrid Genotypes .....	20
Discussion .....	22
Genetic Variation in the Hybrids .....	22
Distribution of Genomic Classes .....	29
Evolutionary Significance of Hybrids .....	33
Conclusions .....	35
References .....	36
Appendix A: Localities and Specimens Examined .....	41
Appendix B: Allele Designations and Erythrocyte Sizes .....	44
B-1: <i>Ambystoma jeffersonianum</i> .....	46
B-2: <i>Ambystoma laterale</i> .....	47
B-3: <i>Ambystoma laterale-jeffersonianum</i> .....	51
B-4: <i>Ambystoma</i> (2) <i>laterale-jeffersonianum</i> .....	55
B-5: <i>Ambystoma laterale-(2) jeffersonianum</i> .....	66
B-6: <i>Ambystoma</i> (3) <i>laterale-jeffersonianum</i> .....	76
B-7: <i>Ambystoma</i> (2) <i>laterale-(2) jeffersonianum</i> .....	77
B-8: <i>Ambystoma laterale-(3) jeffersonianum</i> .....	78

## ABSTRACT

Salamanders of the genus *Ambystoma* present a confusing assemblage of species and hybrid combinations. In eastern North America, hybrids are thought to have originated from up to four distinct species and can be diploid, triploid, tetraploid, or pentaploid. We reviewed the history of hybrid *Ambystoma* in North America and examined 1002 specimens from 106 sites in New York and New England to determine the present northeastern distribution of bisexual species and hybrid combinations. Because of the complex nature of the hybrids, it was important to use several methods to characterize the diploid bisexual species and the hybrids. Area measurements of erythrocyte nuclei provided a rough indication of ploidy but could not be used to distinguish genotypes. Electrophoretic examination of tissue isozymes when combined with chromosome counts proved to be useful for documenting the presence of *Ambystoma jeffersonianum* (JJ), *A. laterale* (LL), and six distinctive hybrid combinations (LJ, LLJ, LJJ, LLJJ, LLLJ, LJJJ; each letter

designates a genome) in New York and New England. The two bisexual species were found to be mostly homozygous for alternate allozymes of several isozymes, which provided heterozygous patterns that were used to characterize the hybrids. Electrophoretic phenotypes were also used to demonstrate recombination and genome replacement among the hybrids. Seventy percent of the salamanders we examined were hybrid females, and few sites contained only diploid bisexuals. Triploid hybrids are much more common than diploid or tetraploid hybrids (70% of specimens). Our survey yielded very few hybrid males (0.8% of specimens). Hybrid populations are maintained by breeding with one of the diploid bisexual species, and the hybrid offspring are the result of gynogenetic or hybridogenetic reproduction. These hybrid salamanders represent a unique and dynamic genetic system in which the hybrids interact with the species through exchange of nuclear genomic DNA but maintain a hybrid cytoplasm.

## INTRODUCTION

Paramount to the establishment of accurate ranges of any species is the accurate identification of individuals. Salamanders of the

genus *Ambystoma* are among the best studied amphibians in North America, but the distribution of some species is still problematic.

The largest obstacle to accurate identification is the fact that, over large areas of eastern North America, hybrid individuals are often more commonly encountered than are the bisexual species. Compounding the practical problems of identification, this complex hybrid system presents challenges of a historical or theoretical nature, including (1) determining the mechanisms of origin and perpetuation of the various hybrid forms and individual populations; (2) establishing a system of nomenclature that is accurate and informative, yet practical; and (3) determining the ecological and evolutionary implications of the existence of the hybrid populations.

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#### HISTORICAL OVERVIEW

The first recognition of the existence of hybrids among mole salamanders, at the time referred to as members of the *Ambystoma jeffersonianum* complex, is attributed to Clanton (1934). He observed populations in southern Michigan to consist of dark and light individuals. Among the dark salamanders, there were equal numbers of males and females, but the light salamanders were invariably female. Dark males mated with both dark and light females. The dark females gave rise to dark males and females; the light females gave rise to only light females. Clanton hypothesized that the light form was a hybrid between a western (or northwestern) race and an eastern race of *A. jeffersonianum*. He also suggested that the populations that contained hybrids were headed for extinction because the proportion of males would be expected to continually decline as a consequence of the all-female reproduction of the light form. Clanton's conclusions were largely ignored in Bishop's (1941; 1947) comprehensive publications on salamanders and in Comeau's (1943) account of *Ambystoma tremblayi*, which he described as a new species from Cap Rouge, Québec. Minton (1954) encountered populations in Indiana similar to those studied by Clanton (1934) in

Michigan. *Ambystoma laterale* Hallowell, 1856, was resurrected by Minton as representing Clanton's "dark form." The other species Minton implicated in the production of the hybrids in Michigan and in Indiana was *A. jeffersonianum* (Green, 1827). *Ambystoma laterale* Hallowell, 1856, was considered a subspecies of *A. jeffersonianum* by Cope (1867), but he later (1889) treated *A. laterale* as a synonym of *A. jeffersonianum*. Minton reiterated Clanton's extinction hypothesis and attributed the "Clanton Effect" to some of his ponds in Indiana. Minton also invoked a post-Pleistocene prairie peninsula extension as a possible causal agent allowing for speciation of *A. laterale* and *A. jeffersonianum* from a putative common ancestor.

Uzzell (1963) added a new dimension to the complex by revealing that the hybrids were naturally occurring triploids and that the triploids could be associated with either of the two diploid species, *A. jeffersonianum* or *A. laterale*. This observation was expanded by Macgregor and Uzzell (1964), who measured the DNA content in erythrocytes of diploid and triploid members of the *A. jeffersonianum* complex and examined lampbrush chromosomes of diploid and triploid individuals. These data were used to hypothesize a gynogenetic reproductive mechanism for the triploid females: an endomitotic event elevated the triploid oocyte to a hexaploid level so that, following meiotic reduction, the ova were triploid. Further, because the observed chiasmata in the lampbrush bivalents were believed to be between daughter chromosomes only (as a result of the endomitotic event), the ova were genetically identical to the female. In gynogenesis, the sperm serves only to stimulate development of the triploid ovum and is not incorporated into the genome of the offspring.

In 1964, Uzzell published a comprehensive treatment of the *A. jeffersonianum* complex, morphologically characterized the two diploid species (*A. jeffersonianum* (Green, 1827) and *A. laterale* Hallowell, 1856), and formally recognized the hybrids as two triploid species (*Ambystoma platineum* Cope, 1867, and *A. tremblayi* Comeau, 1943). *Ambystoma platineum* was deemed to be the triploid associated with *A. jeffersonianum*, and *A. tremblayi* was Clanton's (1934) light

form and the triploid associated with *A. laterale*. Uzzell (1964) justified his contention that the two triploids warranted species recognition based on his belief that they were unique populations that were genetically and morphologically distinct. He cited the precedence of the formal recognition and naming of the all-female fish species *Poecilia formosa*, which was also of hybrid origin (Hubbs and Hubbs, 1932). The names of the salamander triploids were chosen from the available names whose original descriptions matched Uzzell's morphological examinations and distributions.

Uzzell (1964), in a lengthy discussion, rejected Minton's (1954) suggestion that the prairie peninsula had been a barrier that isolated the ancestral diploid populations. Rather, he postulated, the isolation was a consequence of Pleistocene glaciation such that *A. laterale* emerged from a refuge somewhere near the "driftless area" of Wisconsin and that *A. jeffersonianum* migrated north from a refugium south of the ice sheet and within its current range. The hypothetical hybridization event was realized when the ice sheet retracted less than 10,000 years ago. At that time, *A. laterale* dispersed in a northeasterly direction to meet the northwesterly dispersion of *A. jeffersonianum*.

The first electrophoretic study (Uzzell and Goldblatt, 1967) of the complex examined serum proteins from all four of its members. The results showed clear distinction between *A. laterale* and *A. jeffersonianum*. The triploids had complementary patterns: staining intensities suggested that the triploid genome of *A. platineum* contained two sets of chromosomes from *A. jeffersonianum* and one from *A. laterale*, whereas the genome of *A. tremblayi* contained the reciprocal sets of chromosomes. Uzzell and Goldblatt (1967) postulated that hybridization preceded triploidy: a putative diploid hybrid disposed to producing unreduced eggs would, upon backcrossing to one or the other of the two parental species, give rise to the two kinds of triploids, which would also produce unreduced eggs. The newly formed triploids would maintain their genetic integrity by using sperm from sympatric males merely to stimulate development (gynogenesis). Uzzell and Goldblatt (1967) maintained, based on

Macgregor and Uzzell's (1964) evidence, that any genetic variation in the triploids would be limited to mutation.

The discovery of diploid and triploid unisexual *A. laterale* × *Ambystoma texanum* hybrids on Lake Erie's Bass Islands (Downs, 1978) demonstrated that other species of *Ambystoma* were involved in hybridization events. Unlike the *A. jeffersonianum* complex hybrids, most of the hybrids that Downs found on the Bass Islands were diploids, but triploids did occur. Because neither parental species was found on one of the islands, Downs suggested that the hybrids, at least on that island, were reproducing by parthenogenesis. Electrophoretic patterns of serum proteins supported the morphological data that the diploid unisexuals contained chromosome complements of *A. laterale* and *A. texanum* and the triploids showed a double dose of *A. texanum*. Downs also detected polymorphism of serum proteins in the hybrids on the Bass Islands and in mainland bisexual diploids of *A. texanum* and *A. jeffersonianum* as well as morphological variability in the hybrids that were sympatric with *A. texanum*. This led Downs to suggest that the reproduction of *A. laterale* × *A. texanum* hybrids followed neither a strictly parthenogenetic nor a strictly gynogenetic mode. Downs was the first to suggest hybridogenesis as an alternative reproductive mode for salamanders similar to that described by Schultz (1969) for unisexual fish.

Other studies of serum proteins (Servage, 1979; Weller and Menzel, 1979) showed that genetic variability also existed in the triploids and diploids of the *A. jeffersonianum* complex that could not be easily attributed to mutational events within the triploid clones. Despite these findings, Weller and Menzel (1979) adhered to the hypothesis that gynogenesis was the only mode of reproduction used by *A. jeffersonianum* complex triploids and cited Uzzell (1970) and Cuellar (1976), who echoed the earlier findings of Macgregor and Uzzell (1964).

In an attempt to elucidate the evolutionary significance of polyploidy in amphibians and reptiles, Bogart (1980) reviewed the available evidence and found many concepts to be problematic and often founded on tenuous data in the case of *Ambystoma* polyploids.

These involved significant evolutionary implications, such as (1) males deriving no evolutionary benefit from choosing to mate with triploid females; (2) the possibility of heterozygote advantage over homozygotes in changing environments; (3) the significance of duplicated genes or elevated quantities of DNA; (4) the possibility of reconstituting diploid bisexuals; and (5) the significance of gynogenesis, parthenogenesis, and hybridogenesis as possible reproductive strategies.

A karyological analysis of all the members of the *A. jeffersonianum* complex was done by Sessions (1982), who conclusively demonstrated the chromosome composition of the two triploids proposed by Uzzell (1963) and Uzzell and Goldblatt (1967). Sessions' detailed study used C-bands and cold-induced secondary constrictions to differentiate *A. laterale* and *A. jeffersonianum* chromosomes, and he was able to demonstrate recognizable WZ/ZZ (female/male) sex chromosome heteromorphism in *A. laterale*. Sessions assumed gynogenesis and constructed a model that could be used to explain the evolution of the triploids from the diploids. His model was essentially the same as that presented by Uzzell and Goldblatt (1967), but he added possible pathways for production of tetraploids and even diploids from the allotriploids. The embellishments were included to account for his discovery of two sizes of zygotene-pachytene nuclei in the ovaries of newly transformed individuals of both triploid types and to account for his finding of tetraploid and diploid larvae among the offspring of triploid individuals. In an independent study, diploids and triploids in single egg masses were also encountered by Bogart (1982) in natural breeding ponds in southern Ontario.

Tetraploid trihybrids were subsequently reported that involved *A. platineum* and either *A. texanum* (Morris and Brandon, 1984) or *Ambystoma tigrinum* (Morris, 1985). The fourth species to be added to the list of hybridizing species, *A. tigrinum*, was also found to produce hybrids with *A. laterale* × *A. texanum* hybrids. Kraus (1985a) described a new species, *Ambystoma nothogenes*, that was a trihybrid combination, *A. laterale* × *A. texanum* × *A. tigrinum*, from Kelleys Island in Lake Erie. The justification Kraus used for

naming the hybrid was similar to that used by Uzzell (1964) for naming *A. platineum* and *A. tremblayi*. Kraus believed the new species to be all female, fertile, and self-reproducing. Kraus (1985b) was the first to document the existence of *A. laterale* × *A. texanum* hybrids on the Ohio and Michigan mainland. Bogart et al. (1987) examined the Kelleys Island salamanders and demonstrated that *A. nothogenes* was not monophyletic and that this hybrid combination could be created on a yearly basis whenever a *A. laterale* × *A. texanum* hybrid obtained sperm from *A. tigrinum*. The genome composition of hybrid *Ambystoma* in most of the studies described so far was inferred based on morphological examination, erythrocyte area determinations, and identification of sympatric males of one of the bisexual species, according to the criteria provided by Uzzell (1964, 1967a, 1967b, 1967c, 1967d). Uzzell (1969) ascribed parental status to *A. laterale* for *A. tremblayi* hybrids and to *A. jeffersonianum* for *A. platineum* hybrids based on the general allopatric association of the two diploid species and on his observation that males were most likely to court female triploid hybrids that had the genotype most similar to their own. These criteria were not sufficient to identify all the hybrid combinations (Downs, 1978; Bogart, 1982; Kraus, 1985a, 1985b; Bogart et al., 1985, 1987; Zeyl and Lowcock, 1989; Lowcock et al., 1991). Erythrocyte area provides only an indication of ploidy, and not genotype or genome constitution. Even the ploidy estimation is not absolute, because erythrocyte area variation exists and there is considerable overlap between ploidy classes (Austin and Bogart, 1982). Morphology provides an indication of hybrid status, but the genomic contribution and ploidy are usually not obvious and must be confirmed by other methods (Zeyl and Lowcock, 1989).

Multi-locus starch gel electrophoresis added a significant dimension in attempts to unravel the genomic constitution of hybrid *Ambystoma* (Servage, 1979; Bogart, 1982, 1989; Bogart et al., 1985, 1987; Nyman et al., 1987; Lowcock, 1989). All four species of *Ambystoma* that may be involved in different hybrid combinations are largely homozygous and possess more than one species-specific allele. Heterozygous patterns in a hybrid re-

veal the genomes possessed and the intensity of the patterns usually reveals the specific number of genomes involved. It is possible, using this method, to document the hybrid composition of larvae and even eggs (Bogart, 1982). By 1989, 18 hybrid combinations were listed by Vrijenhoek et al. (1989). These included diploid, triploid, tetraploid, and pentaploid genomic combinations of two or three of the four parental species (*A. jeffersonianum*, *A. laterale*, *A. texanum*, or *A. tigrinum*).

With the advent of molecular techniques that distinguish maternal inheritance through an examination of the mitochondrial genome, it was expected that information could finally be obtained concerning single versus multiple origins of hybrid combinations as well as the identification and possible origin of the females responsible for a hybrid. Examination of mitochondrial DNA (mtDNA) clearly resolved the origin and parentage of parthenogenetic lizards of the genera *Cnemidophorus* (Densmore et al., 1989a, 1989b; Moritz et al., 1989a, 1989b), *Heteronotia* (Moritz, 1991), and *Lacerta* (Moritz et al., 1992). All known hybrid combinations of *Ambystoma* (Vrijenhoek et al., 1989) include at least one *A. laterale* genome and, based on that observation, *A. laterale* was a primary suspect as the original female involved in the hybridization events. However, Kraus (1989) and Kraus and Miyamoto (1990) studied mtDNA using restriction fragment length polymorphism (RFLP), and their data suggested that the ancestral species was most similar to *A. texanum*, even in hybrids that did not contain *A. texanum*! A subsequent study by Hedges et al. (1992) examined sequences of the mtDNA gene, cytochrome *b*, and found the various hybrid combinations had sequences that were similar to themselves and unlike that of any of the four parental species of *Ambystoma*. Although unexpected, these mtDNA results answered some very important questions and altered many previously held notions. The apparent disparity between the RFLP and cytochrome *b* sequence analyses may be explained by examination of sequence data that Hedges et al. provided for all four bisexual species and a suitable out-group (*Plethodon*). It is clear from the evolutionary tree (fig. 2 in Hedges et al., 1992)

that *A. texanum* diverged from an early ancestral lineage after the divergence of the main hybrid cluster but before the lineage that gave rise to both *A. jeffersonianum* and *A. laterale*. Thus, *A. texanum* would align more closely with the hybrids than would the other three species. Using *Plethodon* as an outgroup, Hedges et al. (1992) rooted the evolutionary tree and defined the clusters in a relative time frame. They estimated the time of origin of the main unisexual hybrid lineage to be about 4 million years ago and suggested that this predated the evolution of any of the four species of *Ambystoma* that could possibly be a maternal ancestor for the hybrids.

The discrepancy between the data derived from multi-locus electrophoresis and those obtained from mtDNA analyses was not previously observed in any organism. A hybrid *Ambystoma* can possess nuclear genes that are entirely consistent with those of contemporary bisexual species, but this same hybrid possesses mitochondrial genes that differentiate it from the bisexual species at possibly a generic or even family level of divergence. The uncoupling of nuclear and mitochondrial genomic evolution can be explained best by the unique breeding system of the hybrids. Through time, the nuclear genome must both incorporate and eliminate chromosomes as it maintains residence in an ancient cytoplasm.

## REPRODUCTIVE MECHANISMS

Knowledge of the mode of reproduction of hybrid *Ambystoma* is essential to improving our understanding of the relationship of the hybrids with the bisexual diploid species. Although unisexuality has evolved independently several times in fish, amphibians, and reptiles (reviewed in Dawley and Bogart, 1989), it must still be considered rare and unusual among vertebrates.

Three methods of zygote production (outlined in Dawley and Bogart, 1989) are known in unisexual vertebrates: (1) parthenogenesis, (2) gynogenesis, and (3) hybridogenesis. Parthenogenesis and gynogenesis are genetically identical mechanisms that produce offspring containing only maternal genes. Gynogenetic reproduction requires sperm from a male of a bisexual species to stimulate the eggs to de-

velop, but the genome of sperm is not incorporated into the nucleus of the zygote. Gynogenetic reproduction occurs in the hybrid fish *Molleneisa formosa* (Turner, 1982; Monaco et al., 1984). In hybridogenetic reproduction, the sperm is incorporated, but upon maturity of the offspring, the paternal genome is eliminated in a meiotic or pre-meiotic event while the rest of the hybrid's genome is passed on to future generations, usually in an unaltered state. This reproductive mechanism may be termed hemicalonal and is seen in poeciliopsid fishes (Schultz, 1969, 1977) and in frogs of the European *Rana esculenta* complex (Berger, 1977; Graf and Pelez, 1989).

Egg formation in unisexual organisms is of two types: (1) apomixis, formation of eggs by mitotic division, and (2) automixis, eggs being the product of meiosis, which is usually preceded by doubling of the chromosomes. Unisexual vertebrates have been found that are apomictic or automictic (Monaco et al., 1984; Dawley and Bogart, 1989). The theoretical consequences of apomixis and automixis are very different. Over considerable time, ameiotic apomixis may lead to completely heterozygous clones because mutant genes cannot be eliminated by meiotic crossover events. Alternatively, automictic clones may tend to become homozygous because, over time, any crossover events that produce a homozygous condition would eliminate alternate alleles that could never be retrieved, although mutations would still occur.

Macgregor and Uzzell (1964) examined lampbrush chromosomes derived from the oocytes of triploid hybrids of *Ambystoma*. They found that most eggs contained 42 meiotic bivalents. The data show the triploid hybrids to be automictic and indicate that the chromosome number doubles before the diplotene stage in the first meiotic prophase. Uzzell and Goldblatt (1967) assumed that crossovers would occur only between the identical sister chromosomes that resulted from the prediplotene duplication event. Thus, these hybrids, and other automictic unisexual vertebrates with prediplotene endomitosis, could undergo meiotic crossover that would not produce genetic variation. The consequences of sister chromosome crosso-

vers in automictic individuals would be genetically the same as apomixis.

Asher and Nace (1971) predicted the evolutionary consequences of gynogenetic reproduction in *Ambystoma*. They applied data tabulated by Macgregor and Uzzell (1964), which included a few quadrivalent associations, and they assumed a 10,000-year period for the hybridization event and initiation of gynogenesis as hypothesized by Uzzell (1964) and Uzzell and Goldblatt (1967). Asher and Nace's (1971) model demonstrated that, even under strict gynogenesis (or parthenogenesis), the triploid salamanders should not be heterozygous. To explain the disparity between their expectations (homozygosity) and observations of Uzzell and Goldblatt (1967) of fixed heterozygosity in natural populations, Asher and Nace suggested that the predicted theoretical consequences might not be fully realized in populations subjected to the forces of natural selection (Asher, 1970; Asher and Nace, 1971). Selection against individuals carrying mutant alleles would reduce heterozygosity below expectation in an apomictic population. Conversely, a selective advantage for heterozygous individuals would produce a lower-than-expected proportion of homozygous individuals in an automictic population.

#### NOMENCLATURE

The application of species names to hybrids and unisexual clones is an open invitation for critics who adhere to various species concepts to expound on the utility of such designations. Simpson's (1951, 1961) concept of a "unitary evolutionary role" obviously applies to both bisexual and unisexual organisms. Monophyletic origin and a stable genetic trajectory have been the criteria used to justify the use of formal names among parthenogenetic lizards (Maslin, 1968; Cole, 1985) and gynogenetic salamanders (Uzzell, 1964; Kraus, 1985a). Many authors have criticized formal recognition and naming of *Ambystoma* hybrids (Lazell, 1968, 1971; Gilhen, 1974; Cook and Gorham, 1979; Bogart, 1980; Bogart and Licht, 1986; Lowcock et al., 1987). Dubois and Günther (1982) decided that new systematic categories should be established for particular ani-

mal forms that could not be adequately covered under a biological species concept. They coined the word klepton (from the Greek word for thief) to be used for forms that stole gametes from other species for reproductive purposes. Gynogenetic and hybridogenetic vertebrates are two different kinds of kleptons. Additionally, a klepton always occurs sympatrically with one or more species from which it arose by hybridization and the grouping may even include more than one klepton. Dubois and Günther (1982) suggested that such groups be called synkleptons. Under their system, *Ambystoma jeffersonianum* would be the abbreviated name for *Ambystoma* (synkl. *jeffersonianum*) *jeffersonianum* (Green, 1827) and *Ambystoma platineum* would represent *Ambystoma* (synkl. *jeffersonianum*) kl. *platineum* (Cope, 1867), or *Ambystoma* kl. *platineum*. The evidence for strict gynogenesis in hybrid *Ambystoma* was reviewed by Bogart and Licht (1986) and was found to be tenuous. In laboratory crosses (Bogart et al., 1989), individual triploid hybrid females produced offspring that (1) were triploid and did not incorporate the sperm nucleus (gynogenetic); (2) were triploid and did incorporate the sperm nucleus (hybridogenetic); or (3) were tetraploid and did incorporate the sperm nucleus, especially at elevated temperatures. These data corroborate the cytological observations made by Sessions (1982), the mixed ploidy and electrophoretic variation observed in single egg masses by Bogart (1982), the "clonal" variation observed by Downs (1978), the electrophoretic variation and ploidy variation observed by Bogart et al. (1985, 1987) among offspring from genetically identified individual females from Pelee and Kelleys Islands, and the multiple origin of triploid hybrids (Lowcock and Bogart, 1989). The accumulated data reveal *Ambystoma* hybrids to be a dynamic and unique system. Reproduction of hybrid females is often gynogenetic but may be hybridogenetic. The sperm nucleus can also be incorporated in unreduced eggs to elevate the ploidy to triploid (from a diploid hybrid), tetraploid (from a triploid hybrid), and even pentaploid (presumably from a tetraploid hybrid). The nucleus in a hybrid may contain genomic contributions from four (currently recognized) different species. Each

genome resides in the nucleus for an undetermined length of time or number of generations and may be introduced to newly incorporated genomes during a sexual episode. The nucleus resides in cytoplasm that is not the same as the cytoplasm of any of the contemporary species whose genomes are constituents of the nucleus. The hybrid's cytoplasm has been inherited matrilineally as a consequence of the all-female reproduction of the hybrids. Under such a system, the number of possible kleletons would reflect the permutations and combinations of the four genomes that could reside in diploid, triploid, tetraploid, or pentaploid nuclei. Ignoring rare pentaploid hybrids, which would substantially increase the number of possible combinations, more than 50 combinations may presently occur in nature. Many of these hypothetical combinations may not be viable, such as *Ambystoma laterale-texanum-tigrinum-tigrinum* (Bogart et al., 1987), but even a preliminary listing of the 18 known combinations (Vrijenhoek et al., 1989) shows that kleletonic designations are neither desirable nor useful for elucidating the genetic and evolutionary problems posed by this fascinating system.

Lowcock et al. (1987) suggested an informal descriptive system for hybrid *Ambystoma* that was used by Schultz (1969) to describe the genetic composition of hybridogenetic unisexual fish of the genus *Poeciliopsis*. Letter designations for species that contributed genomes to hybrids have previously been used for convenience by a number of authors (Uzzell, 1964; Uzzell and Goldblatt, 1967; Downs, 1978; Kraus, 1985a, 1985b; Bogart et al., 1985, 1987): J for *A. jeffersonianum*, L for *A. laterale*, T for *A. texanum*, and Ti for *A. tigrinum*. The proposed name, for example, for *A. platineum* would be *Ambystoma laterale-(2) jeffersonianum*, or LJJ. Dubois and Günther (1982) opposed such designations on several grounds, including unpalatability of compound names, different names for diploid and polyploid hybrids that involved the same hybrid combination, and nomenclatural changes of names that have been in general use. They suggested, for the sake of stability of nomenclature, that names such as *Ambystoma platineum* should be retained but transferred

from the category species to the category kleleton even when the proper evolutionary status and genetic features are known. To avoid compound names, Dubois and Günther formalized Schultz's (1969) names from, for example, *Poeciliopsis 2 monacha-lucida* to the more palatable (in their opinion) *Poeciliopsis* (synkl. *occidentalis*) kl. *duomona-chalucida* Schultz, 1969. We believe it is unlikely that these two systematic categories (or three if Dubois and Günther's suggestion of the additional category subkleleton is accepted) will be widely endorsed in studies of hybrid *Ambystoma*. Our nomenclature follows that suggested by Lowcock et al. (1987), rejecting the use of formal species names for the hybrid combinations in this salamander complex and using, instead, informal names based on ploidy and electrophoretically determined genomic contributions of *A. laterale* and *A. jeffersonianum* in hybrids. Such informal references allow efficient and effective communication without suggesting that these hybrids are species.

#### NEW YORK AND NEW ENGLAND *AMBYSTOMA*

The current ranges of *A. jeffersonianum*, *A. laterale*, and hybrids in New York and New England are based on relatively few individuals from few localities. Uzzell (1964) identified *A. jeffersonianum* from Massachusetts (3 males, 5 females), New Jersey (1 male), New York (36 males, 1 female), and Vermont (1 male); and *A. laterale* from Maine (15 males), Massachusetts (8 males, 3 females), New York (31 males, 1 female), and Vermont (1 male). The discrepancy in sex ratios that Uzzell reported was based on the premise that males could be identified with certainty from preserved museum collections but females of "pure" species could be confused with hybrid females. Uzzell used erythrocyte size to distinguish the diploid species from triploid hybrids. The relatively few females were identified from live individuals using morphology and erythrocyte size. Uzzell distinguished genome composition based largely on the sympatric associations of the "pure" species: triploid females occurring with *A. jeffersonianum* were regarded as *A. platineum* (LJJ) and those found

with *A. laterale* were *A. tremblayi* (LLJ). Triploid females were found in Massachusetts (8 LJJ, 1 LLJ) and New Jersey (9 LJJ). Later, Anderson and Giacosie (1967) found *A. laterale* in New Jersey.

Electrophoretic and karyotypic analyses provide more accurate methods for determining ploidy and genomic content in hybrids. Nyman et al. (1988) used diagnostic alleles to document the occurrence of *A. laterale* and two triploid combinations (LLJ and LJJ) in New Jersey. These methods allowed Lowcock (1986, 1989) to examine distribution patterns in eastern North America and Klemens (1993) to define the general distribution of the complex in New England and eastern New York.

Our study was originally undertaken to provide a more complete survey of *A. laterale*, *A. jeffersonianum*, and their hybrids in New England. As the data accumulated, new hybrid combinations and different ploidies were encountered. Additional collecting in certain populations and geographic regions was deemed necessary to test new theoretical questions. Some of the same specimens have been used in other studies for comparative purposes and to test the utility of karyotypes, erythrocytes, and isozyme electrophoresis in distinguishing different hybrid combinations throughout the range of this complex (Austin and Bogart, 1982; Bogart et al., 1989; Lowcock, 1986, 1989; Hedges et al., 1992; Klemens, 1993).

## MATERIALS AND METHODS

### COLLECTION

Salamanders were collected from breeding ponds early in the spring, crossing roads at night during spring rains, or from under rocks and logs during the day from March through October. A few specimens, collected as eggs or larvae, were raised through metamorphosis at the American Museum of Natural History. Because the area surveyed was large and the salamanders are difficult to collect outside the breeding season, this study was conducted over an 18-year period from 1978 to 1995. One thousand and two individual salamanders from 106 sites (figures 1 and 2; appendix A) were examined. Most

specimens were shipped from New York to Guelph a few days after they were collected. Salamanders were injected with colchicine (0.25–0.75 ml of a 0.1-mg/ml solution) 2 days before they were killed by prolonged anesthesia in a 7% solution of buffered (pH 7.0) tricaine methane sulfonate (MS222). After the tissues were removed, the specimens were preserved and deposited at the American Museum of Natural History (AMNH). A few specimens were also deposited at the Museum of Comparative Zoology (MCZ) and the University of Michigan Museum of Zoology (UMMZ). These specimens are listed by museum number in appendix A.

### ELECTROPHORESIS

Tissues used for electrophoresis were liver (L) and a combination of heart, skeletal muscle, and spleen (HMS). The tissues were removed from freshly killed salamanders and stored with an equal volume of deionized water in 1.5-ml Eppendorf microtubes in an ultracold freezer ( $-70^{\circ}\text{C}$ ). Just prior to electrophoresis, the frozen tissues were ground using a sharp glass rod and centrifuged for 2 minutes in a microfuge. Horizontal starch gel electrophoresis followed the procedures outlined by Selander et al. (1971), Bogart (1982), and Murphy et al. (1996). The buffer systems were described by Selander et al. (1971) and Clayton and Tretiak (1972). Electrophoretic loci for each enzyme system were numbered on the gel from the most anodally migrating locus. As in previous studies (Bogart et al., 1987; Bogart, 1989), alleles or allozymes were designated by their relative mobilities compared with the mobility of the most common allele in *A. laterale*, which was assigned a mobility of 100. The 21 loci that were assayed, buffer systems, and the tissues examined are shown in table 1. These particular loci were chosen because they possess alleles that have been shown to distinguish *A. laterale* from *A. jeffersonianum* and they have been used for the identification and characterization of this complex as well as other *Ambystoma* hybrids in mainland Ontario (Bogart, 1982, 1989) and on Pelee Island (Bogart et al., 1985; Bogart and Licht, 1986) and Kelley's Island (Bogart et al., 1987) in Lake Erie. The number of electro-

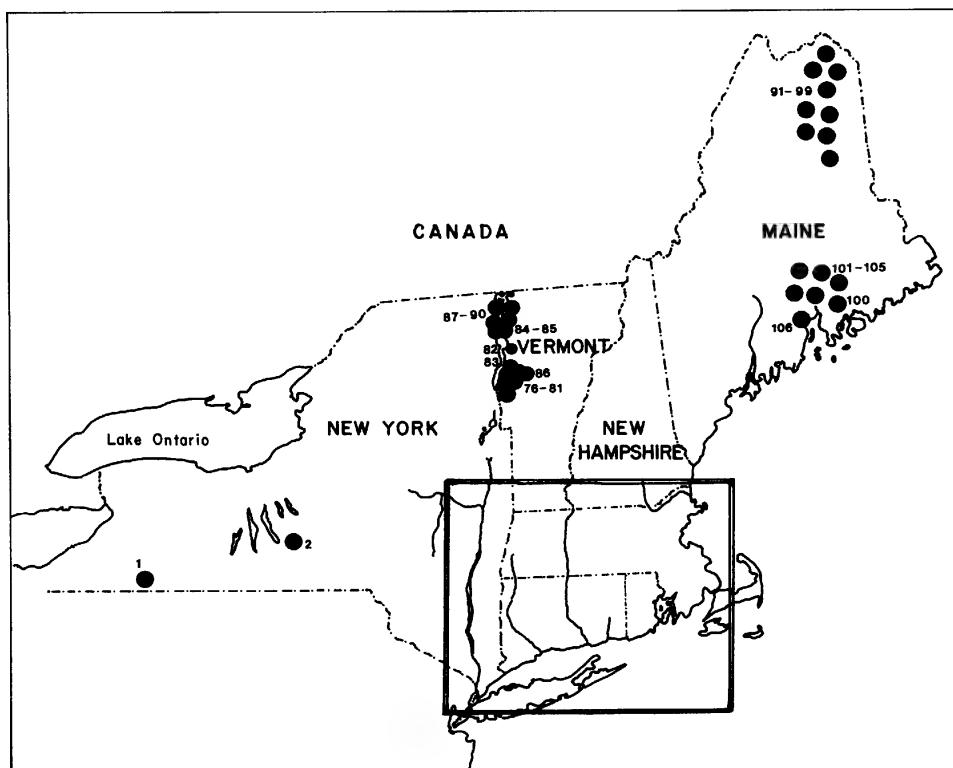


Fig. 1. Collection sites for *Ambystoma jeffersonianum*, *A. laterale*, and hybrids in New York and New England. Specimens examined for each of the numbered sites in figures 1 and 2 are listed in appendix A. The area enclosed in the rectangle is expanded in figure 2.

phoretic loci assayed for individual salamanders varied. Initially, a large number of loci were examined to find alleles important for distinguishing the parental genome combinations in hybrids and to estimate clonal diversity among the hybrids. When it was discovered that only a few loci were necessary to assess the genome contributions in a hybrid (Bogart et al., 1985; Bogart, 1989) and that the hybrids were not strictly clonal (Bogart and Licht, 1986), loci were chosen that were the least expensive to stain and that could be resolved using a single buffer system. Dimeric isozymes were the most useful for visualizing different staining intensities of the bands (dosage), thus enabling assessment of genome composition in polyploid amphibians (Danzmann and Bogart, 1982).

As the study progressed, additional loci were included to test new hypotheses concerning reproductive mechanisms of hybrid *Ambystoma*. Rarely encountered hybrid individuals had homozygous (rather than het-

erozygous) alleles, and some hybrids had unexpected reversed electrophoretic phenotypes (Bogart, 1989). Additional information was required from these individuals for linkage analyses based on hypothesized meiotic mechanisms.

#### BLOOD CELL SIZE

Traditionally (Uzzell, 1964, 1967a, 1967b, 1967c, 1967d; Wilbur, 1976), average erythrocyte area has been the method used to determine ploidy in the *A. jeffersonianum* complex. A small drop of blood was taken from every individual, mixed with saline, and photographed under phase-contrast and bright-field optics. The area of the blood cells was determined using a sonic digitizer on a rear projection of the negatives (Austin and Bogart, 1982). Six blood cells from each individual were measured to obtain an average erythrocyte area. Although the blood cell size varied and there was overlap between

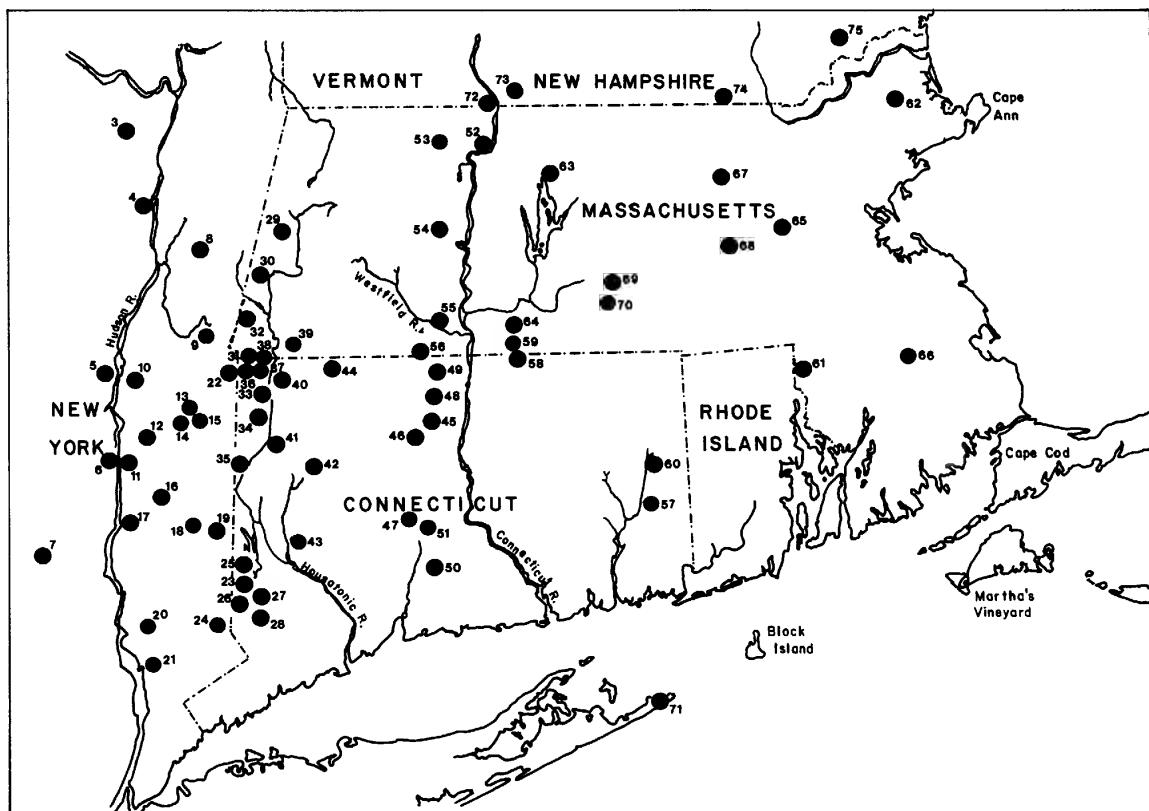


Fig. 2. Collection sites in southern New England and southeastern New York.

ploidy classes, these data provided the first clear indication of diploid and tetraploid hybrids that might not have been distinguished using only the electrophoretic patterns. For example, a diploid (LJ) and a symmetrical tetraploid hybrid (LLJJ) would have the same electrophoretic phenotypes, but the corresponding blood cell areas would be 600–700  $\mu\text{m}$  (LJ) and 1100–1400  $\mu\text{m}$  (LLJJ).

#### CHROMOSOMES

The intestines including the cloaca, were dissected from all individuals. This tissue was immersed in deionized water for 15 minutes and then fixed in 3:1 ethanol: acetic acid and stored at  $-20^\circ\text{C}$ . Chromosomes were obtained from the gut epithelial tissue using procedures outlined by Kezer and Sessions (1979) and Sessions (1982). Because the chromosomes of *A. laterale* and *A. jeffersonianum* are virtually indistinguishable (Taylor and Bogart, 1990), chromosomes could not be used to assign genomic constitution in the

hybrids. The chromosome numbers were, however, positive proof of ploidy in the hybrids, and these data were required if a discrepancy existed between the electrophoretic genotype and the blood cell area (e.g., the diploid LJ and symmetrical tetraploid LLJJ hybrids). Diploid, triploid, and tetraploid hybrids were confirmed by chromosome counts.

#### SITE DESIGNATIONS

Because we were interested in knowing the bisexual and hybrid associations in breeding populations, we analyzed the individuals that were expected to breed in the same location. These salamanders occupy ponds only for breeding. Thus, in most cases, individuals could be assigned to distinct breeding ponds because they were collected as adults or larvae in the pond or as newly transformed juveniles at the edge of the pond. Sometimes, however, individuals were collected crossing the road at night and could

TABLE 1  
Presumptive Structural Gene Loci Examined in *Ambystoma*

Locus (abbreviation) <sup>a</sup>	EC no. <sup>b</sup>	Tissue <sup>c</sup>	Gel <sup>d</sup>
Acid phosphatase (AcPh-2 or ACP-2)	1.1.3.2	L	2
Aconitase hydratase (Acon-1 or ACOH-1)	4.2.1.3	L	1
Alcohol dehydrogenase (Adh)	1.1.1.1	L	2
Creatine kinase (Ck-2)	2.7.3.2	HMS	1
Esterase (Est-1 <sup>e</sup> )	2.1.1.-	L	3
Aspartate aminotransferase (Got-1 or AAT-1 <sup>f</sup> )	2.6.1.1	HMS	2
Got-2		L	2
Isocitrate dehydrogenase (Idh-1 or ICD-1)	1.1.1.42	HMS	1
Lactate dehydrogenase (Ldh-1)	1.1.1.27	HMS	1
Ldh-2		HMS	1
Malate dehydrogenase (Mdh-1)	1.1.1.37	HMS	1
Mdh-2		HMS	1
Mdh-3		L	1
Malic enzyme (Me-1 or MDHP-1)	1.1.1.40	L	1
Mannose-6-phosphate isomerase (MPI or PGDH)	5.3.1.8	L	1
Phosphoglucose isomerase (Pgi or GPI)	5.3.1.9	HMS	2
Phosphoglucomutase (Pgm-1)	2.7.5.1	L	1
Pgm-2		L	1
Superoxide dismutase (Sod-1)	1.15.1.1	L	1

<sup>a</sup>Loci examined were those that were found to be most useful for distinguishing the species and hybrids. The abbreviations and synonyms are those used in this and previous electrophoretic studies of vertebrates.

<sup>b</sup> Standardized numbering system established by the Nomenclature Committee of the International Union of Biochemistry (IUBC, 1984).

<sup>c</sup> Tissues used to resolve the enzyme systems were liver (L) or a combination of heart, skeletal muscle, and spleen (HMS).

<sup>d</sup> The electrophoretic conditions for the gels were (1) amine-citrate gel buffer and tray buffer pH 6.5 (Clayton and Tretyak, 1972), run for 3 hours at 250 volts; (2) Tris-citrate gel buffer pH 6.7 and tray buffer pH 6.3 (Selander et al., 1971), run for 3–4 hours at 150 volts; (3) citric acid gel buffer pH 9.2 and borate tray buffer pH 8.2 (Poulik gel; Selander et al., 1971), run for 4–5 hours at 250 volts.

<sup>e</sup> Substrate alpha-naphthyl acetate.

<sup>f</sup> Got (glutamate oxaloaceto-transaminase) instead of AAT because previous studies on *Ambystoma* used this earlier enzyme designation.

not be assigned to a particular breeding pond. Additionally, salamanders were found at different locations entering into extensive swamps that may be partitioned into subpopulations. These factors required the use of site designations based on the assumption that individuals from the same site shared a potentially common breeding area. A site may or may not be equated with a breeding population. In some instances, we combined individuals into sites when they were collected from extensive yet ecologically similar and contiguous habitat. Some other collections that were geographically very close were treated as separate sites when the sites were distinctly separated by habitat and, in some instances, elevation. Habitat was found by Klemens (1993) to be an important factor

partitioning the two bisexual species. He found that topography played an important role in determining wetland type, which in turn influenced the distribution of the bisexual species. This was most apparent in areas of close contact, where breeding ponds of the two bisexual species were separated by as little as 100 meters. We grouped our sites into drainage basins because Klemens (1978, 1993) demonstrated that there were significant differences in the herpetofauna of New England's drainage basins, as a result of post-Pleistocene dispersion of amphibians and reptiles into the interior of New England.

#### GENOTYPE ANALYSES

Because the unisexuals are mostly fixed heterozygotes and their mode of reproduction

involves gynogenesis and hybridogenesis, population genetic models that are based on random interbreeding do not apply to the unisexuals. As well, models that are based on strictly clonal reproduction, facultative parthenogenesis, or recurrent hybridization are not useful without modification and additional information concerning the mode of reproduction of individual female hybrids. When unisexual hybrids occur in a population, they usually outnumber individuals of the bisexual species. If the sample obtained from a site contained neither *A. laterale* nor *A. jeffersonianum* individuals, we suspected that the sample was too small to have encountered either or both of these species. To obtain some measure of the presence or influence of the bisexual species, we calculated the genomic percentage of *A. laterale* at each site, obtaining values from 0% (all *A. jeffersonianum*) to 100% (all *A. laterale*). Under this scheme, triploid LLJ would be 66.7%. If the average percentage of all the individuals from a site was below 50% then *A. jeffersonianum* was assumed to be the sperm donor for that population even though the sample of salamanders may not have included *A. jeffersonianum*.

## RESULTS

Based on chromosome counts and diagnostic alleles, the specimens included *Ambystoma laterale* (LL), *A. jeffersonianum* (JJ), and six hybrid combinations: *A. laterale-jeffersonianum* (LJ), *A. laterale-(2) jeffersonianum* (LJJ), *A. laterale-(3) jeffersonianum* (LJJ), *A. (2) laterale-jeffersonianum* (LLJ), *A. (2) laterale-(2) jeffersonianum* (LLJJ), and *A. (3) laterale-jeffersonianum* (LLLJ). Representatives of the eight genotypes are included in figures 3 and 4. The sites where these genomic types were found as well as the percentages of the bisexual species and hybrid combinations are included in table 2. More than half of the specimens examined were triploid hybrid females, and few sites contained only diploid bisexuals. Eight hybrid males were found among the "unisexual" hybrid females.

Ten of the 106 sites were found to contain only *A. laterale* without the sympatric association of hybrid LLJ (100% L; table 2).

Some of these sites may prove not to be pure *A. laterale* populations because they were only represented by one or two individuals. Montauk, Long Island (Site 71,  $n = 43$ ), had the largest sample of *A. laterale* with no hybrid associates. The Quinebaug River Valley of eastern Connecticut (Site 57,  $n = 11$  and Site 60,  $n = 17$ ) and Attleboro, Bristol County, Massachusetts (Site 61,  $n = 12$ ), are also pure *A. laterale* sites based on more than 10 specimens from each site. Even though the sample size was small at West Bridgewater, Plymouth County, Massachusetts (Site 66,  $n = 4$ ), it is close to the other *A. laterale* sites and may also represent a pure *A. laterale* site. Site 65 (Sudbury, Middlesex County, Massachusetts) is represented by a single *A. laterale* but, because this locality lies between Essex and Worcester counties, Massachusetts, which have a variety of hybrid genotypes allied with *A. laterale*, it is probable that Site 65 also is not pure *A. laterale*. Individuals of *A. laterale* were collected at Site 90, Birdland, Grand Isle County, Vermont ( $n = 2$ ) and Sites 93 and 99, Aroostook County, Maine (each represented by  $n = 1$ ). However, because all the other sites in Grand Isle and Aroostook counties have LLJ, additional samples would be required before we could conclude that any of these sites contain only *A. laterale*. *Ambystoma jeffersonianum* was found at only three sites without hybrids. These sites were Dryden, Tompkins County, New York (Site 2,  $n = 3$ ); Buckland, Franklin County, Massachusetts (Site 53,  $n = 1$ ); and Winchester, Cheshire County, New Hampshire (Site 73,  $n = 1$ ). The low sample sizes preclude designating any of these sites as pure *A. jeffersonianum*.

Diploid LJ hybrids were found at 21 sites and were associated with either *A. laterale* or *A. jeffersonianum*. Except for Site 68, where the entire sample consisted of a lone diploid hybrid, all sites where diploid hybrids were found also had triploid LLJ or LJJ hybrids. Tetraploid hybrids were found at 27 sites. Except for Sites 38, 78, and 86, where the entire sample consisted of only one or two tetraploids, all of the other sites with tetraploid hybrids also had triploid LLJ or LJJ hybrids. The eight male hybrids were of three different hybrid combinations and three ploidies: LJ (2), LLJ (5), and LLJJ (1). The

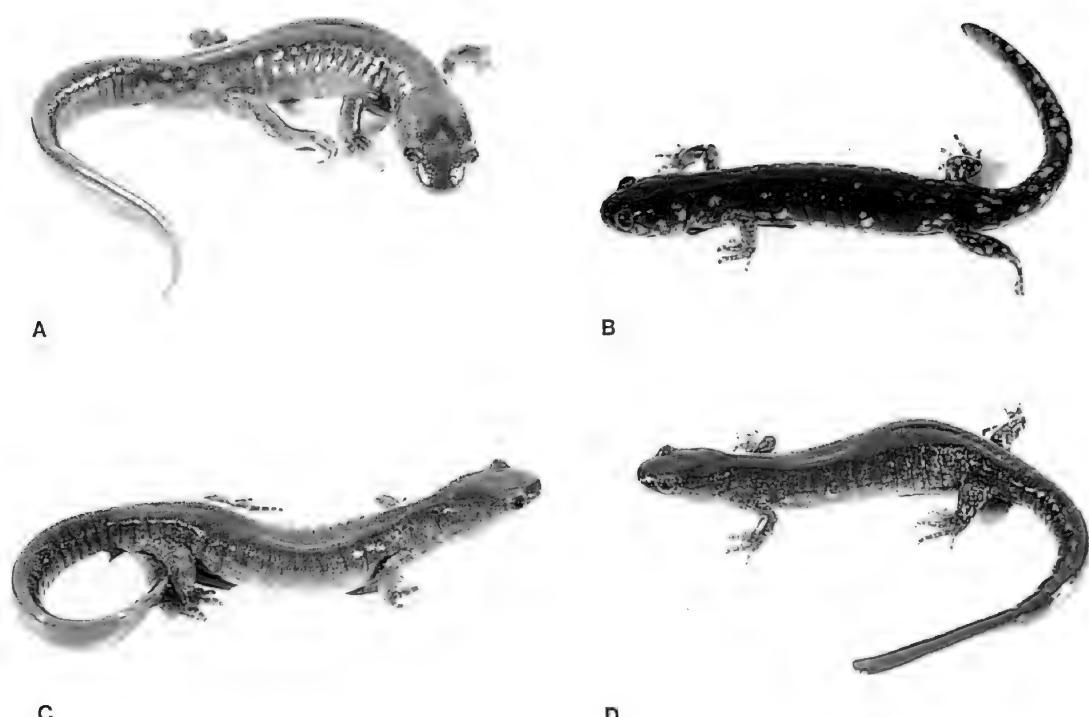


Fig. 3. Representative salamanders. Diploid species: (A) *Ambystoma jeffersonianum* (JJ), AMNH 140490; (B) *A. laterale* (LL), AMNH 139459. The two common triploid hybrids: (C) *A. laterale*-(2) *jeffersonianum* (LJJ), AMNH 139449; (D) *A. (2) laterale-jeffersonianum* (LLJ), AMNH 139453.

only two males found at Site 40 ( $n = 23$ ) were a diploid LJ hybrid and a tetraploid LLJJ hybrid. The second LJ hybrid male was the only LJ individual found at Site 70 ( $n = 28$ ). Two of the four males found at Site 89 ( $n = 26$ ) were LLJ hybrids; the other two males at that site were *A. laterale*. Single male LLJ hybrids came from Sites 17 ( $n = 15$ ), 62 ( $n = 22$ ), and 80 ( $n = 23$ ), and these three sites also had normal *A. laterale* males.

#### CHROMOSOMES

When this study was initiated, an attempt was made to obtain chromosomes from every individual, but this proved to be unrealistic. The time involved in obtaining countable chromosomes varied considerably among individuals. When ploidy was revealed in the electrophoretic analyses and the specimen had an erythrocyte area determination that was within a normal ploidy range, chromosome counts simply confirmed these other data. With the exception of a few specimens that died before they were processed,

tissue for chromosomes was collected and maintained for all specimens. The preserved gut provided ample epithelial tissue to prepare a large number of slides ( $>100$  for adult *A. jeffersonianum*). The cloacal region yielded the greatest number of dividing cells, but it was usually necessary to prepare and scan several slides for each individual before preparations were found that contained suitable chromosome spreads. A suitable spread possessed all the chromosomes from one nucleus in a reasonably compact area on the slide and had few overlapping chromosomes. Because the primary purpose for observing chromosomes in this study was simply to obtain accurate ploidy designations for individuals, slides were prepared and scanned until the chromosomes in at least two suitable spreads could be counted and photographed. The haploid chromosome number of 14 yields 28 chromosomes in a diploid, 42 in a triploid, and 56 in a tetraploid (figure 5). In this cursory examination, karyotypic analyses were not performed. If an individual ap-

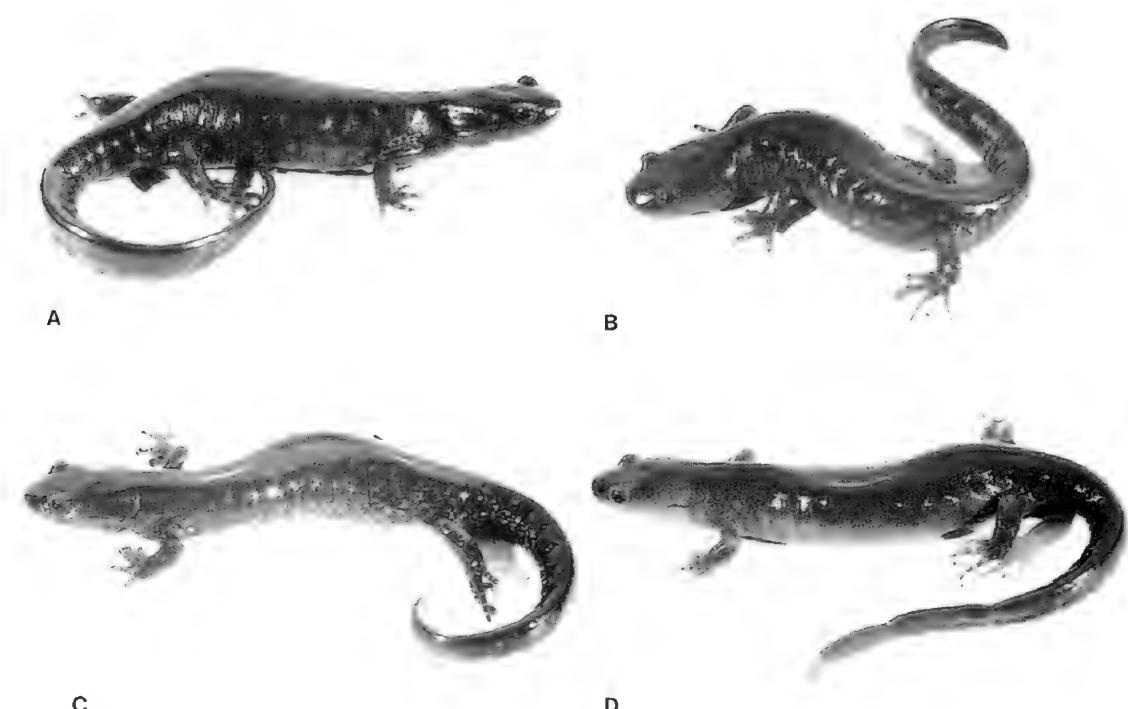


Fig. 4. Representative salamanders. (A) Diploid hybrid *A. laterale-jeffersonianum* (LJ), AMNH 129284. Tetraploid hybrids: (B) *A. laterale-(3) jeffersonianum* (LJJJ), AMNH 129432; (C) *A. (2) laterale-(2) jeffersonianum* (LLJJ), AMNH 121988; and (D) *A. (3) laterale-jeffersonianum* (LLLJ), AMNH 139434.

peared to be an aneuploid or a mosaic, additional preparations were scanned. No abnormal counts were observed consistently in any salamander during the present investigation. A concerted effort was made to confirm the ploidy of diploid and tetraploid hybrids. Also, chromosome counts were required from those individuals with an electrophoretic pattern that did not correspond with the erythrocyte area determinations.

#### AREA OF ERYTHROCYTES

Figure 6 compares the blood cells from diploid, triploid, and tetraploid salamanders. The average area measurements of six erythrocytes for each individual are included with the electrophoretic results in appendix B. These data are summarized for each genomic combination in table 3. Data from blood cell analyses were not obtained from a few individuals (— or ? in appendix B) because these individuals died before being processed and possessed abnormal blood cells or there

were accidental problems with the processing of the negatives. If the area determinations were unusually high or unusually low, chromosomes were examined to confirm the ploidy for the genomic combination. When this study was initiated, we did not expect to find diploid or tetraploid hybrids, and the blood cell data provided the first indication of these "new" hybrid combinations. Once tetraploids were known to exist in natural populations, an individual that was determined to have a chromosome number that was obviously above the diploid level could not automatically be considered a triploid. Some sites had salamanders that seemed to have greater variation in erythrocyte area than at other sites, and a few individuals of the "pure" species had blood cell sizes in the triploid range. Juvenile specimens usually demonstrated greater variation in erythrocyte areas. The range of erythrocyte areas of the triploids spanned all ploidy groups, but the very high and very low values were derived

from relatively few individuals (reflected in the standard deviation). Chromosomes were examined to confirm ploidy when the blood cell data were anomalous.

#### ELECTROPHORESIS

Twenty loci were examined for most of the salamanders, which provided information on the genomic constitution of the individuals from each site. The electrophoretic analyses for *A. laterale*, *A. jeffersonianum*, and all six genomic combinations of hybrids (LJ, LJJ, LLJ, LJJ, LLJJ, LLLJ) are included in appendix B. These data were used to calculate gene frequencies, which are summarized in table 4 for the eight genomic groups (*A. laterale*, *A. jeffersonianum*, and the six hybrids) over all 106 sites. Only one locus (6Pgd) was found to be fixed ( $p = 1.000$ ) for a common allele in all of the salamanders. Four of the polymorphic loci (Ck-1, Mdh-2, Mdh-3, Pgm-2) were homozygous for a common allele in most specimens and from all sites, but rare alternate alleles were found in a small number of individuals from relatively few sites.

**DIAGNOSTIC ALLELES:** From previous studies (Bogart, 1982, 1989; Bogart et al., 1985), several electrophoretic loci have been identified that are homozygous for alternate alleles in *A. laterale* and *A. jeffersonianum*. These alleles identify the two species and can be used to determine the genomic constitution of a hybrid based on the intensity (dosage) of the alternate alleles. The specimens in this study were not subjected to detailed morphological examination, but the identification based on the diagnostic alleles corresponded to the expected, distinctive morphology of the two bisexual species. No specimen that was identified by electrophoresis as *A. laterale* had an *A. jeffersonianum* phenotype and vice versa. The same diagnostic alleles that were found in Ontario (Bogart et al., 1985) are also present and diagnostic in the New York and New England populations, which represent the most southern populations of *A. laterale* and the most northeastern populations of *A. jeffersonianum*.

Five loci were found to be fixed ( $p = 1.000$ ) for alternate alleles in the two species:

Ck-2, Est-1, Got-1, Idh-1, and Sod-1. An additional five loci could be used to separate most individuals: Acon-1, Got-2, Ldh-1, Mdh-1, and Mpi. Representative electrophoretic gels are shown in figures 7 and 8 for Ck, Got, Ldh, Mdh, and Sod. These enzyme systems can distinguish all of the genotypes in the present study. The "*jeffersonianum*" Acon-1<sup>79</sup> allele was present in *A. laterale* individuals from Sites 17, 50, and 71, and the "*laterale*" Acon-1<sup>100</sup> allele was found in *A. jeffersonianum* from Sites 14, 22, 52, and 76. All six of the *A. jeffersonianum* examined for Acon-1 from Site 76 were homozygous for the "*laterale*" allele, but this allele was found in a heterozygous condition only in individual specimens from three other sites (14, 22, and 52). Got-2 was fixed for the "*jeffersonianum*" allele, Got-2<sup>-180</sup>, in all *A. jeffersonianum*; no *A. laterale* had the "*jeffersonianum*" allele, but one *A. laterale* from Site 79 was heterozygous for a very rare allele, Got-2<sup>-50</sup>. None of the *A. jeffersonianum* had the "*laterale*" Ldh-1<sup>100</sup> allele, but one *A. jeffersonianum* from Site 76 was heterozygous for a rare Ldh-1<sup>115</sup> allele. Of the three individuals of *A. laterale* examined from Site 1, one was homozygous for the "*jeffersonianum*" Ldh-1<sup>188</sup> allele and one was an Ldh-1<sup>100/88</sup> heterozygote. Mdh-1 was fixed in *A. laterale* (Mdh-1<sup>100</sup>) and fixed for an alternate allele (Mdh-1<sup>176</sup>) in all *A. jeffersonianum* except two Mdh-1<sup>176/100</sup> heterozygotes from Site 1.

Eight loci were chosen as the diagnostic loci for the genetic analyses of the hybrids: Ck-2, Got-1, Got-2, Idh-1, Ldh-1, Mdh-1, Mpi, and Sod-1. Even though Est-1 appeared to be fixed for alternate alleles in the two species, unexpected variation was encountered among the hybrids. By staining for esterase on a low pH gel system, we discovered that the alleles that appeared on the high pH gel represented as many as three separate loci, which rendered examination of band intensity very difficult in the hybrids. Even though Acon-1 appeared to be a good diagnostic locus, the alternate alleles were scattered over a large range of sites in both of the diploid bisexual species. In addition, Acon-1<sup>100</sup> and Acon-1<sup>79</sup> alleles were not separated on the gel sufficiently to provide distinct bands in many of the hybrids, which sometimes made scoring problematic.

TABLE 2  
*Ambystoma laterale*, *A. jeffersonianum*, and Hybrid Genomes from New York and New England

Site	(n) <sup>a</sup>	Males <sup>b</sup>	Genome <sup>c</sup>									%L <sup>d</sup>	
			2n			3n		4n					
			IL	LJ	JJ	LLJ	LJJ	LLLJ	LLJJ	LJJJ			
1	(5)	0	3	1	—	1	—	—	—	—	—	81.8	
2	(3)	1	—	—	3	—	—	—	—	—	—	0.0	
3	(39)	0	4	21	—	13	—	1	—	—	—	62.4	
4	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
5	(22)	2	—	—	2	—	20	—	—	—	—	31.2	
6	(15)	1	—	—	1	—	14	—	—	—	—	31.8	
7	(10)	1	3	4	—	2	—	1	—	—	—	70.8	
8	(1)	0	—	—	—	1	—	—	—	—	—	33.3	
9	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
10	(2)	0	—	—	—	—	2	—	—	—	—	33.3	
11	(12)	0	—	—	—	—	9	—	1	2	—	33.3	
12	(20)	0	—	2	—	—	16	—	—	2	—	30.3	
13	(22)	5	—	8	7	—	7	—	—	—	—	29.4	
14	(7)	1	—	—	2	—	5	—	—	—	—	26.3	
15	(2)	0	—	—	—	1	1	—	—	—	—	50.0	
16	(2)	0	—	—	—	2	—	—	—	—	—	66.7	
17	(14)	4*	4	3	—	6	—	1	—	—	—	77.8	
18	(30)	1	2	10	—	17	—	1	—	—	—	64.6	
19	(7)	0	—	—	—	—	7	—	—	—	—	33.3	
20	(12)	0	—	3	1	—	8	—	—	—	—	34.4	
21	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
22	(12)	0	—	4	1	—	7	—	—	—	—	35.5	
23	(24)	0	—	9	—	13	1	—	—	1	—	59.4	
24	(2)	1	2	—	—	—	—	—	—	—	—	100.0	
25	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
26	(3)	0	—	1	—	2	—	—	—	—	—	62.5	
27	(12)	0	—	—	2	—	10	—	—	—	—	29.4	
28	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
29	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
30	(12)	1	—	5	1	—	6	—	—	—	—	36.7	
31	(5)	0	—	—	1	—	4	—	—	—	—	28.8	
32	(14)	4	—	—	4	—	9	—	—	1	—	25.6	
33	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
34	(4)	1	—	—	1	—	3	—	—	—	—	27.3	
35	(6)	3	—	1	5	—	—	—	—	—	—	8.3	
36	(26)	1	2	—	—	24	—	—	—	—	—	68.4	
37	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
38	(1)	0	—	—	—	—	—	1	—	—	—	75.0	
39	(1)	0	—	—	—	—	1	—	—	—	—	33.3	
40	(23)	2*	3	3	—	15	—	1	1	—	—	67.6	
41	(12)	0	—	—	—	—	7	—	—	—	5	29.3	
42	(15)	0	2	—	—	12	—	1	—	—	—	70.4	
43	(17)	3	—	—	4	—	11	—	—	—	2	26.5	
44	(12)	1	—	1	3	—	8	—	—	—	—	28.1	
45	(21)	2	3	—	—	18	—	—	—	—	—	70.0	
46	(33)	2	—	—	5	—	26	—	—	—	2	29.2	
47	(4)	1	—	—	3	—	1	—	—	—	—	11.1	
48	(3)	0	1	—	—	2	—	—	—	—	—	75.0	
49	(5)	1	1	1	—	3	—	—	—	—	—	69.2	
50	(30)	3	24	—	—	6	—	—	—	—	—	90.9	

TABLE 2  
(Continued)

Site	(n) <sup>a</sup>	Males <sup>b</sup>	Genome <sup>c</sup>									%L <sup>d</sup>
			2n			3n			4n			
			IL	LJ	JJ	LLJ	LJJ	LLLJ	LLJJ	LJJJ		
51	(23)	0	—	—	—	—	23	—	—	—	—	33.3
52	(13)	3	—	—	7	—	6	—	—	—	—	18.8
53	(1)	0	—	—	1	—	—	—	—	—	—	0.0
54	(3)	0	—	—	—	—	3	—	—	—	—	33.3
55	(6)	3	—	—	3	—	3	—	—	—	—	20.0
56	(12)	0	—	—	—	—	12	—	—	—	—	33.3
57	(11)	3	11	—	—	—	—	—	—	—	—	100.0
58	(5)	0	—	—	—	5	—	—	—	—	—	66.7
59	(1)	0	—	—	—	1	—	—	—	—	—	66.7
60	(17)	7	17	—	—	—	—	—	—	—	—	100.0
61	(12)	6	12	—	—	—	—	—	—	—	—	100.0
62	(22)	2*	5	1	—	15	—	1	—	—	—	72.1
63	(3)	0	—	—	—	—	3	—	—	—	—	33.3
64	(8)	0	—	—	—	8	—	—	—	—	—	67.7
65	(1)	0	1	—	—	—	—	—	—	—	—	100.0
66	(4)	3	4	—	—	—	—	—	—	—	—	100.0
67	(13)	1	2	3	—	8	—	—	—	—	—	67.6
68	(1)	0	—	1	—	—	—	—	—	—	—	50.0
69	(3)	0	—	—	—	3	—	—	—	—	—	67.7
70	(28)	12*	22	1	—	4	—	1	—	—	—	90.3
71	(43)	24	43	—	—	—	—	—	—	—	—	100.0
72	(1)	0	—	—	—	—	1	—	—	—	—	33.3
73	(1)	1	—	—	1	—	—	—	—	—	—	00.0
74	(6)	0	2	—	—	2	—	—	—	2	—	80.0
75	(16)	1	4	—	—	12	—	—	—	—	—	74.3
76	(24)	6	—	1	8	1	13	—	—	—	1	26.6
77	(7)	2	2	—	—	5	—	—	—	—	—	73.7
78	(2)	0	—	—	—	—	—	—	1	1	—	62.5
79	(9)	3	3	—	—	2	—	—	4	—	—	78.6
80	(23)	3*	9	—	—	11	—	3	—	—	—	77.8
81	(4)	0	—	—	—	3	—	1	—	—	—	69.2
82	(11)	3	3	—	—	5	—	3	—	—	—	75.8
83	(7)	3	5	—	—	2	—	—	—	—	—	87.5
84	(9)	1	2	—	—	6	—	1	—	—	—	73.1
85	(1)	0	—	—	—	1	—	—	—	—	—	67.7
86	(1)	0	—	—	—	—	—	—	1	—	—	75.0
87	(15)	1	4	—	—	10	—	—	1	—	—	73.8
88	(11)	3	4	—	—	7	—	—	—	—	—	75.9
89	(26)	4*	2	—	—	24	—	—	—	—	—	68.4
90	(2)	1	2	—	—	—	—	—	—	—	—	100.0
91	(6)	0	1	—	—	5	—	—	—	—	—	70.6
92	(2)	0	—	—	—	2	—	—	—	—	—	66.7
93	(1)	1	1	—	—	—	—	—	—	—	—	100.0
94	(9)	2	4	—	—	5	—	—	—	—	—	78.3
95	(10)	0	—	—	—	10	—	—	—	—	—	66.7
96	(16)	1	8	—	—	8	—	—	—	—	—	80.0
97	(3)	0	—	—	—	3	—	—	—	—	—	66.7
98	(4)	1	1	—	—	2	—	1	—	—	—	70.0
99	(1)	0	1	—	—	—	—	—	—	—	—	100.0
100	(2)	0	—	—	—	2	—	—	—	—	—	66.7

TABLE 2  
(Continued)

Site	(n) <sup>a</sup>	Males <sup>b</sup>	Genome <sup>c</sup>								%L <sup>d</sup>
			2n			3n		4n			
IL	LJ	JJ	LLJ	LJJ	LLLJ	LLJJ	LJJJ				
101	(2)	0	—	—	—	2	—	—	—	—	66.7
102	(4)	0	1	—	—	3	—	—	—	—	72.7
103	(1)	0	—	—	—	1	—	—	—	—	66.7
104	(2)	0	—	—	—	2	—	—	—	—	66.7
105	(2)	0	1	—	—	1	—	—	—	—	80.0
106	(1)	0	—	—	—	1	—	—	—	—	66.7
Total (1002)	142	231	84	66	319	256	25	6	15		
Percentage		23.05	8.38	6.59	31.84	25.55	2.50	0.60	1.50		
Percent males		14.17			Percent hybrids		70.36			Percent triploids	57.38
Percent diploid species		29.64			Percent hybrid males		0.80			Percent tetraploids	4.59

<sup>a</sup> Sites and individual specimens are enumerated and described in appendix A.

<sup>b</sup> An \* indicates the finding of rare hybrid males at the site.

<sup>c</sup> Electrophoretically characterized genotypes of diploid, triploid, and tetraploid salamanders: *Ambystoma laterale* genome (L) and *A. jeffersonianum* genome (J).

<sup>d</sup> The percentage of *Ambystoma laterale* genomes (%L) is calculated from the individuals collected at each site.

RARE ALLELES: Mdh-2<sup>34</sup> was the only rare allele ( $p < 0.05$ ) found in several of the genomic groupings. This allele was missing in two of the three tetraploid groups (LJJJ and LLJJ); these two groups had the smallest sample sizes. Other rare alleles were encountered only in a few individuals from a few sites (appendix B; table 4). Most of the rare alleles found in *A. jeffersonianum* were the common alleles in *A. laterale*, except in one specimen of *A. jeffersonianum* from Site 76 that was an Ldh-1<sup>115/88</sup> heterozygote. The Ldh-1<sup>115</sup> allele was not found in any other specimen in this study. The same *A. jeffersonianum* specimen was a Pgm-2<sup>100/75</sup> heterozygote; the rare Pgm-2<sup>75</sup> allele was also found in two LLJ triploids (Site 77 and 88) and one LJJ triploid (Site 5). A single specimen of *A. laterale* from Site 79 was a Got-2<sup>-100/-50</sup> heterozygote; no other specimen was found with a Got-2<sup>-50</sup> allele.

Some of the hybrid combinations possessed rare alleles that were not found in the diploid bisexual species. One of the diploid LJ hybrids (Site 68) was an Ldh-2<sup>100/55</sup> heterozygote; this was the only Ldh-2<sup>55</sup> allele found. One LLJ hybrid was a Pgm-2<sup>125/125/100</sup> heterozygote and was the only specimen in this study that possessed the Pgm-2<sup>125</sup> allele.

Other rare alleles included Ck-1<sup>80</sup>, which was found only in a heterozygous condition in LJJ hybrids (Sites 6, 11, 13, 20, 30, and 32); Got-1<sup>110</sup>, which was found in a heterozygous condition in LLJ hybrids (six from Site 23 and one from Site 26) and in one LLJJ hybrid from Site 23; Mdh-1<sup>135</sup>, which was found only in LLJ hybrids from Site 69 (all three specimens from that site were heterozygous for Mdh-1<sup>135</sup>); Mdh-3<sup>-160</sup>, which was found in one LLJJ tetraploid from Site 40 and in one LLLJ tetraploid from Site 62; and Me-1<sup>60</sup>, which was found only in one LJJ hybrid (Site 56).

HOMOZYGOSITY AND REVERSALS IN HYBRID GENOTYPES: For some loci and in some sites, the diagnostic alleles used to identify the constituent genomes in hybrids did not occur in the expected frequencies. For example, *A. jeffersonianum* and *A. laterale* are homozygous for alternate Got-1 alleles (Got-1<sup>79</sup> and Got-1<sup>100</sup>, respectively). An LJ hybrid, thus, would be expected to have a frequency of 0.500 for each allele, an LLJ would have a frequency of 0.667 for Got-1<sup>100</sup>, and an LJJ would have a frequency of 0.333 for the same allele. Slight deviations from these expected frequencies are evident in table 4 for many of the diagnostic alleles. Some of the

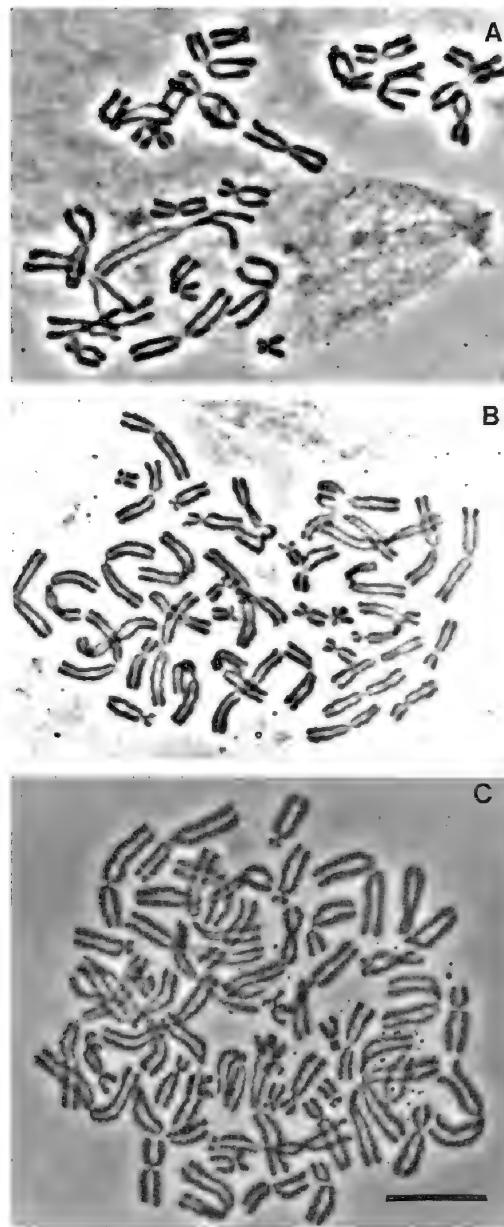


Fig. 5. Chromosome preparations from diploid ( $2n = 28$ ) (A), triploid ( $3n = 42$ ) (B), and tetraploid ( $4n = 56$ ) (C) specimens. Scale bar = 20  $\mu\text{m}$  for all panels.

deviations result from rare alternate alleles when a locus has more than two alleles. Most of the deviations, however, result from a few hybrid individuals that demonstrated unexpected reversals and homozygous genotypes.

The numbers of homozygous genotypes found in the various hybrid genomic groups

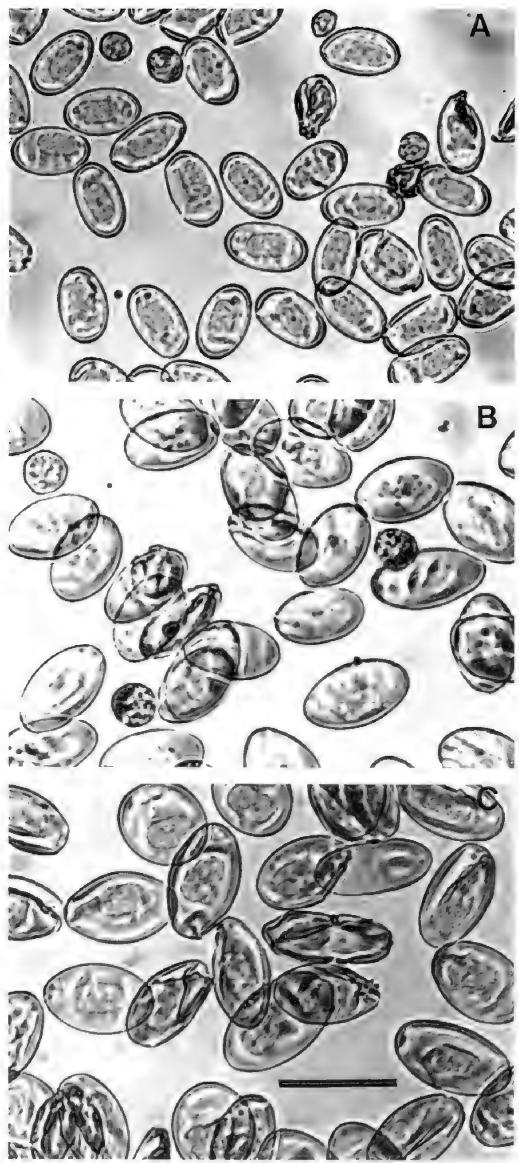


Fig. 6. Erythrocyte preparations from diploid (A), triploid (B), and tetraploid (C) specimens of *Ambystoma*. Scale bar = 50  $\mu\text{m}$  for all panels.

are shown in table 5. Some alleles (0.0% in table 5) were never found in a homozygous condition in the hybrids, but almost 7% of the hybrids were homozygous for Ldh-1<sup>100</sup> (Ldh-1 A in table 5). In two sites (23, 40), most of the LLJ hybrids were homozygous for this allele. Sometimes an LLJ hybrid possessed alleles that would be expected to occur in an LJJ hybrid or vice versa. Such reversed genotypes were rarely observed in all

TABLE 3  
Erythrocyte Area Measurements ( $\mu\text{m}^2$ )

Genome <sup>a</sup>	<i>n</i>	Mean $\pm$ SD	Range
<b>Diploids</b>			
IL	201	684.34 $\pm$ 87.97	407–997
JJ	63	727.65 $\pm$ 85.27	589–960
LJ	80	702.54 $\pm$ 64.17	571–883
<b>Triploids</b>			
LLJ	302	1013.00 $\pm$ 129.45	682–1463
LJJ	239	1008.70 $\pm$ 115.42	714–1600
<b>Tetraploids</b>			
LLLJ	25	1239.28 $\pm$ 141.26	1016–1628
LLJJ	6	1105.00 $\pm$ 119.32	1015–1334
LJJJ	15	1268.00 $\pm$ 148.61	982–1614

<sup>a</sup> Genomes include haploid complements of *Ambystoma laterale* (L) and *A. jeffersonianum* (J). The genotypes and ploidy (2n, 3n, 4n) were determined by electrophoresis and chromosome counts.

of the hybrid genomic groups at various sites, but some of these reversals were concentrated in a particular site or geographical region. Seventeen of the 24 LLJ specimens from Site 89 were reversed for Got-2 alleles, and 15 individuals from this site were reversed for Sod-1 alleles. The numbers of triploid individuals that demonstrated reversed genotypes are shown in table 6 (LJJ) and table 7 (LLJ). The percentage of reversed genotypes for each of the diagnostic loci ranged from 0 (Sod-1 in LJJ, table 6) to a high of 27.8% (Ck-2 in LJJ, table 6). Based on the diagnostic loci, the expected frequencies were also not realized among the tetraploid genotypes for some of the diagnostic alleles (appendix B), but the numbers of tetraploid individuals were relatively low and the intensity (dosage) of the alleles was more difficult to assess.

## DISCUSSION

The electrophoretic identification of the salamanders used in this study relied on the fortuitous discovery of a number of loci that possessed alternate alleles in *Ambystoma laterale* and *A. jeffersonianum* and on the extreme homozygosity of the two species. Using the frequency data provided for the 21 loci in table 4, mean heterozygosities of 0.087 and 0.092 were estimated for *A. laterale* and *A. jeffersonianum*, respectively. Ge-

netic distances between the species were also estimated from these data to be 0.951 (Nei, 1978) and 0.627 (Rogers, 1972). These figures are similar to those found by Bogart et al. (1985) for Ontario, where Nei's genetic distance (D) between the species was 1.067 and the mean heterozygosities were 0.053 for *A. jeffersonianum* and 0.110 for *A. laterale*. Schaffer (1984) found average heterozygosities for 13 species of Mexican ambystomatids also to be low (0.096). These figures indicate that species of *Ambystoma* are, for amphibians, unusually homozygous (Nevo, 1978) and that *A. laterale* and *A. jeffersonianum* are distantly related. Our data and the cytochrome *b* data of Hedges et al. (1992), who estimated that these species diverged about 200,000 YBP (years before present), do not support the hypothesis of Uzzell and Goldblatt (1967) that the speciation of these salamanders was a relatively recent (about 10,000 YBP) Pleistocene event. Sarich (1977) suggested that a Nei's D value of 1.0 would approximate a divergence time of 20 million years (20 MYBP), but such an early date (Miocene) is probably unrealistic according to data obtained for other North American salamanders (Highton and Webster, 1976; Wake and Lynch, 1982; Schaffer, 1984). In our investigation, Nei's D is very high because *A. laterale* and *A. jeffersonianum* share very few alleles for the loci that were examined. The purpose of our study was to identify the genomic constitution of hybrids, and thus we concentrated on loci that possessed distinctive alleles in the two species. An examination of a larger number of loci that are not considered "diagnostic" would provide a more realistic genetic distance.

## GENETIC VARIATION IN THE HYBRIDS

The great majority of hybrids examined were fixed heterozygotes for *A. laterale* and *A. jeffersonianum* alleles and contained the expected phenotypes for diploid, triploid, or tetraploid composition across all of the polymorphic loci. Homozygous genotypes (table 5) and reversed genotypes (tables 6, 7) most likely resulted from meiotic events during oocyte formation. Asher and Nace (1971) demonstrated the possible outcome of quad-

rivalent formation in Meiosis I, which would result in homozygosities and reversed genotypes even when quadrivalent formation was very infrequent. In fact, they used data provided by Macgregor and Uzzell (1964) and showed that *Ambystoma* hybrids should be homozygous for many alleles and should have reversed genotypes. Asher and Nace hypothesized that the fixed heterozygosity observed by Uzzell and Goldblatt (1967) could only be maintained over time in this gynogenetic system if there was strong selection against the homozygous and reversed genotypes. Of course, once a homozygous genotype is attained in a gynogenetic hybrid lineage, there would be no possibility of reverting to a heterozygous condition, except by point mutation. Hybridogenesis was not considered by Asher and Nace, but this reproductive method could allow hybrids to break out of a perpetual homozygous lineage.

The hybrids that did demonstrate homozygous genotypes were not randomly distributed among sites or genotypes. Idh-1 and Ldh-1 had the most homozygosity over the largest number of genomic classes (table 5). In some cases, the homozygotes were restricted to one or a few sites: both Idh-1 (A) LJ homozygotes were from Site 23; two of the three LJ hybrids and 14 of 15 LLJ individuals from Site 40 were Ldh-1 (B) homozygotes; all of the LLJ hybrids from Site 58 were Mpi (A) homozygotes; three of the four Sod-1 (D) LLJ homozygotes were from Site 89. In other cases, the homozygotes were scattered among several sites (appendix B). There were more reversed genotypes than homozygous genotypes, and the individuals that possessed these reversed electrophoretic patterns were clumped in a few sites for some reversed alleles and scattered among several sites for other alleles (tables 6, 7). Site 89 contained a large number of LLJ individuals with reversed genotypes for both Got-2 and Sod-1. Considering the rare occurrence of reversed genotypes, it would appear that these two loci reside on the same chromosome and this is an example of linkage. We had the opportunity to examine the inheritance of some alleles at Site 89 because one female from that site laid eggs that developed into larvae and metamorphosed juveniles. The offspring of that female were

examined electrophoretically; results for four loci that demonstrated reversals are compared in table 8. The female had a reversed (from the expected LLJ) phenotype at Idh-1, but all of the triploid and tetraploid offspring had the expected LLJ or LLLJ phenotype. The female had an expected LLJ phenotype for Ldh-1, but one of her 19 offspring had a reversed phenotype. Only about half of the offspring maintained the female's reversed Got-2 and Sod-1 phenotypes; the others reverted to the expected LLJ or LLLJ phenotype. There is also some indication of linkage between Got-2 and Sod-1 as was noted for adults sampled at this site, but linkage must not be complete; in addition, calculations were hampered because mortality was considerable among the eggs and offspring, thus the surviving individuals were probably not a random sampling of the meiotic process. This particular female's eggs were the only ones that developed through metamorphosis among eggs laid by 12 females from Site 89. Even so, of 116 eggs laid by that female, only 30 produced larvae and only 11 transformed. The larvae (L in table 8) were frozen because they were very abnormal or died.

Other individuals at Site 89 and individuals from a few other sites demonstrated reversed patterns at more than one locus. In a small number of cases, determining the genomic group to which an individual belonged was problematic. How many reversed loci could an LLJ individual maintain before it would be classified as LJ? Indeed, if a large number of species-specific alleles that were not examined and that were more significant in evolutionary terms than the so-called "neutral" alleles visualized in our electrophoretic analyses were shuffled in a hybrid's genome, individual hybrids would be genetically distinctive and would carry a variety of *laterale* and/or *jeffersonianum* alleles subject to natural selection.

Several factors might be responsible for the homozygosities and reversals that were observed. According to Asher and Nace (1971), homozygosities and reversals result from the stochastic events of quadrivalent formation in the first prophase of meiosis. The frequency of homozygotes is a function of the frequency of quadrivalent formation and the number of generations that have

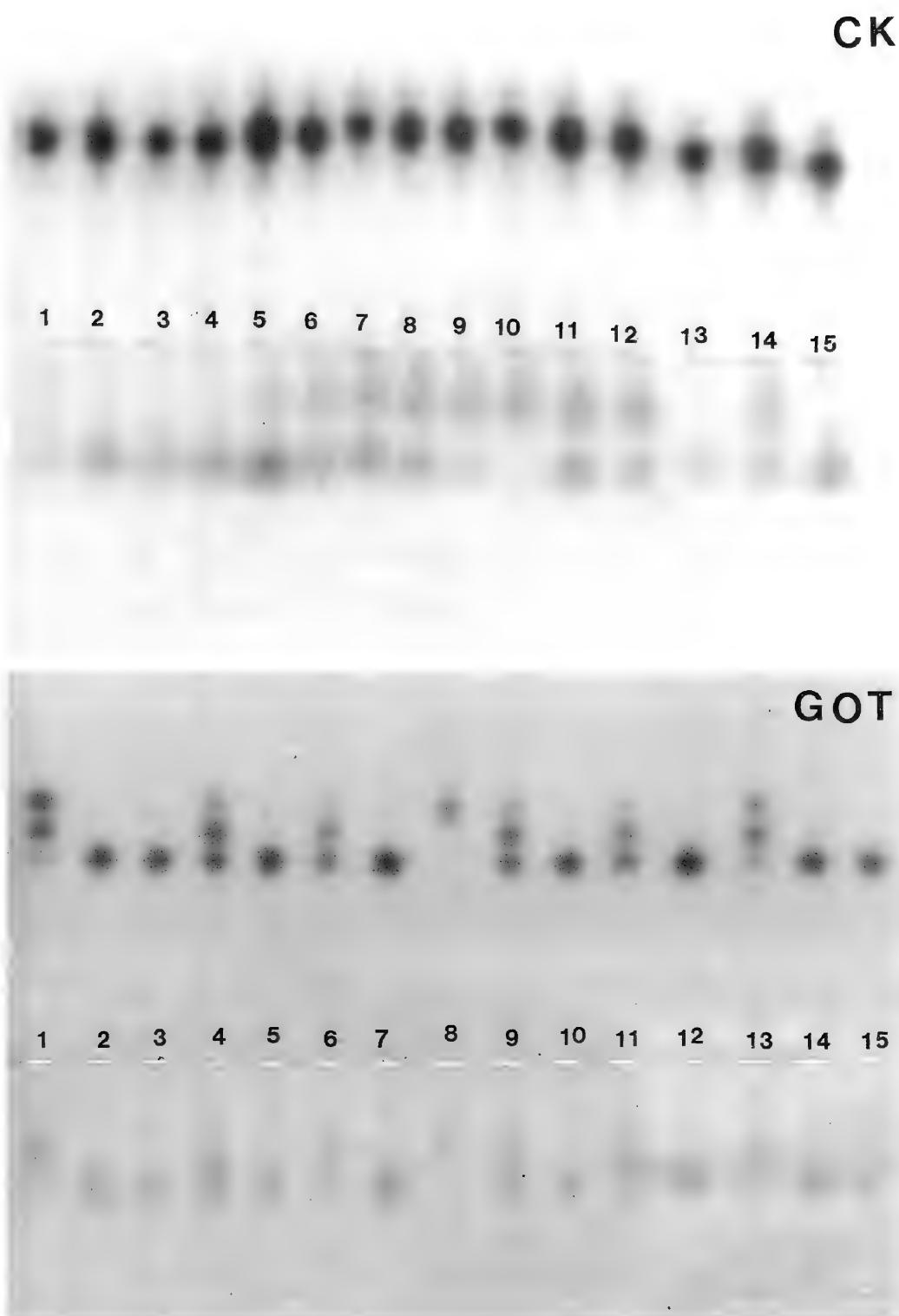


Fig. 7. Electrophoretic gels stained to reveal and compare activity (intensity of staining) of the enzymes creatine kinase (CK) and glutamate oxaloacetotransaminase (GOT) (= aspartate aminotransferase or AAT) in 15 samples. Both enzyme systems possess anodally migrating (toward the top of the

passed since the initial evolution of the gynogenetic hybrid. Taylor (1991) also outlined a model of meiosis that could explain the production of reversals and homozygosities. Based on the fact that the chromosomes double prior to meiotic diplotene, she suggested that reversed and homozygous genotypes could occur simply as a consequence of association of homologous chromosomes in unreduced eggs. For example, a triploid LLJ females primary hexaploid oocyte (LLJJJJ) has two chromosome complements carrying *laterale* genes and four carrying *jeffersonianum* genes. The egg, possessing three of the possible six homologous (or homologous, assuming that the L chromosome and J chromosome are not completely homologous for all loci) chromosomes, may have all *jeffersonianum* genes for a particular locus and be homozygous or have two *laterale* genes and only one *jeffersonianum* gene for a locus and demonstrate a reversed genotype. These models can be used to help explain observed reversal and homozygosities in hybrid females when the offspring are produced by gynogenesis, independent of any sperm contribution. Additionally, as has been shown by Bogart et al. (1989), sperm can be incorporated in both reduced and unreduced hybrid eggs. In a population of LJJ, a female incorporating sperm from an *A. laterale* mating could produce LLJ and LLJJ offspring as well as gynogenetic LJJ offspring. Such sperm incorporation would, however, affect all of the diagnostic loci and reclassify the hybrids (e.g., LJJ, LLJ, LLJJ). The incorporation of a genome is a useful, and the only plausible, mechanism to (1) prevent a homozygous trajectory in an otherwise gyno-

genetic lineage and (2) maintain contemporary genomes in an ancient cytoplasm (Hedges et al., 1992).

The incorporation of alternative genomes (such as a J in eggs produced by LLJ females) would be a likely event in breeding ponds that contain both *A. laterale* and *A. jeffersonianum* or if a hybrid female dispersed from a "*laterale*" to a "*jeffersonianum*" pond. None of the 106 sites were found to contain both diploid bisexual species. Triploid LLJ hybrids were found with LJJ hybrids at only three sites (table 2, Sites 15, 23, 76). Because only two individuals were found at Site 15, it is not possible to predict the possible bisexual species at that site. *Ambystoma jeffersonianum* (Site 14) was found to be the closest diploid species. Diploid bisexual individuals were also not found at Site 23 but, based on the 13:1 ratio of LLJ:LJJ, it is presumed to be an *A. laterale* site. Too few individuals were sampled from adjacent Site 25 (one LJJ) and Site 26 (three hybrids: one LJ and two LLJ), and no diploid bisexuals were found at these sites. At greater distance, *A. laterale* was found at Site 24 and *A. jeffersonianum* was found at Site 27. Male *A. jeffersonianum* were found at Site 76, and the ratio of LLJ:LJJ was 1:13 (table 3). Site 76 is situated close to *A. laterale* sites (75 and 77), so dispersion of a male *A. laterale* or a female LLJ from an adjacent population would be a reasonable assumption for the site. It is of interest that both diploid and tetraploid hybrids were also found in Sites 23 and 76. In fact, the highest frequencies of reversals and symmetric tetraploids (LLJJ) were found in sites where the two species are geographically close or parapatric.

←

figure) and cathodally migrating (toward the bottom of the figure) isozymes designated as Ck-1 or Got-1 and Ck-2 or Got-2, loci, respectively. Ck-1 shows no variation among the compared samples and is designated as monomorphic for allele A or, in terms of mobility, Ck-1<sup>100</sup>. Heterozygosity in the hybrid genotypes is evident at Ck-2, Got-1, and Got-2. *Ambystoma jeffersonianum* is monomorphic for Ck-2 B (sample 10), whereas *A. laterale* is monomorphic for Ck-2 A (samples 1–4, 13, and 15). The patterns for the hybrids are AAAB (sample 5), AAB (samples 6, 7, and 11), ABB (samples 9, 12, and 14), and AB or AABB (sample 8). A clearer resolution of ploidy is presented in the staining pattern of the dimeric enzyme GOT, where the heterodimer (middle band) is obvious. The samples stained for GOT activity are *A. jeffersonianum* (JJ) (samples 2, 3, 5, 7, 10, and 12–14), *A. laterale* (LL) (sample 8), LLJ (samples 1 and 13), and LJJ (samples 4, 6, 9, and 11). Got-2 is also dimeric and shows similar dosage but has weak staining intensity on the gel because the tissues sampled were heart, muscle, and spleen; liver samples provide a stronger staining reaction for Got-2.

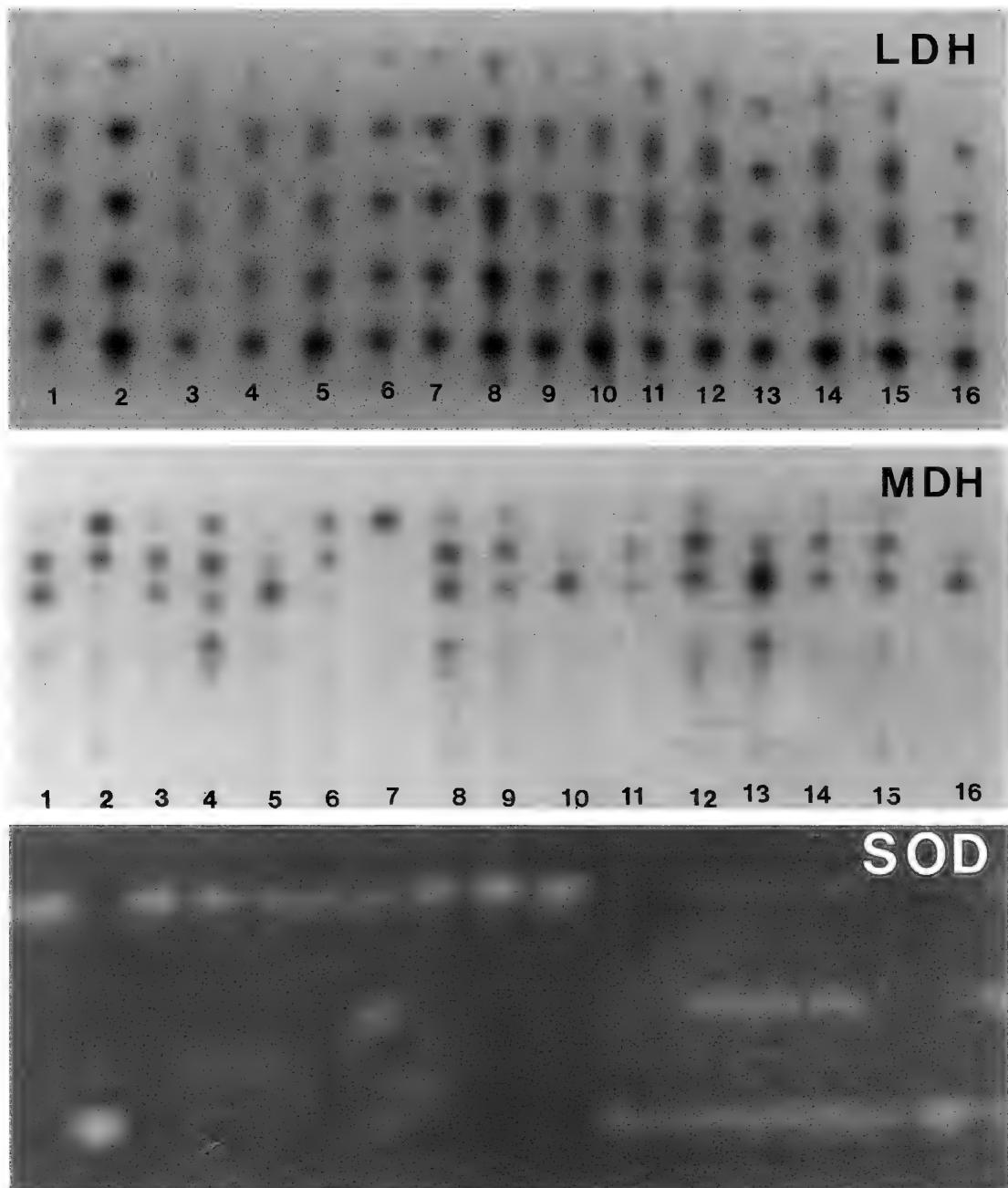


Fig. 8. Electrophoretic gels stained to reveal and compare activity (intensity of staining) of the enzymes lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and superoxide dismutase (SOD) in 16 samples. LDH is a tetrameric enzyme and presents a five-banded pattern in individuals homozygous for Ldh-1 (uppermost homotetrameric band) and Ldh-2 (lowest homotetrameric band) caused by the interaction of four enzyme subunits from two loci. Samples 2, 6, 7, and 16 are *A. laterale* and sample 13 is *A. jeffersonianum*. The heterozygosity of Ldh-1 in the hybrids results in a multi-banded pattern caused by the Ldh-1 homotetrameric bands and the heterotetrameric bands that form between the homodimeric bands. The hybrid samples on the LDH gel are LLJ (samples 1, 4, 5, and 8–10), LJ (samples 3, 12, and 15), and LJ (samples 11 and 14). MDH is a dimeric enzyme with three loci. Mdh-1 (upper portion of the gel) is clear in heart, muscle, and spleen samples, and *A. jeffersonianum* (sample 7) is easily differentiated from *A. laterale* (samples 5, 10, 13, and 16). The hybrids on the MDH gel

Site 89 is the site that yielded the female that gave rise to the offspring described in table 8, and it also produced male *A. laterale* and two triploid hybrid males. We found homozygosity for Sod-1 (D), a *jeffersonianum* allele, at Site 89, as well as more individuals with reversed genotypes than at any other site (table 7). It is conceivable that the two bisexual species could have been sympatric in some localities in the recent past or that hybrids dispersed from a "*jeffersonianum*" to a "*laterale*" breeding pond. Some of the triploid offspring of the Site 89 LLJ female demonstrated genotypes that were reversed from the reversed genotypes of the female to return to the expected genotypes of (BBB) Sod-1, (ABB) Idh-1, and (ABB) Got-2. One individual demonstrated a (BCC) Ldh-1 genotype reversed from the female's (BBC) genotype. An Ldh-1 reversal was also found in one of the 24 Site 89 adult LLJ hybrids (appendix B-4). Judging from the tetraploid genotypes in table 8 and other loci, the sperm that was incorporated must have been from *A. laterale* because, in most cases, the additional alleles in the tetraploids were diagnostic for that species, but the unreduced eggs that gave rise to some tetraploids must have undergone genomic rearrangements to give rise to the observed asymmetric (ABBB) Got-2, (ABBB) Idh-1, and (BBBB) Sod-1 tetraploid genotypes.

We were surprised that there were so few uncommon or rare alleles in the analysis of hybrid genotypes. If gynogenetic reproduction is the common method of the hybrids for many generations, rare alleles descended from an ancestor or derived from mutational events would be maintained in a hybrid "clonal" lineage. In fact, some uncommon alleles that were found demonstrated a non-clonal mode of reproduction. Two western Fairfield County, Connecticut, sites in close proximity, on the divide between the Hudson and Housatonic drainage basins, were unique. Got-1<sup>110</sup> was found in 6 of 13 LLJ

hybrids from the Corner Pond Brook drainage (Site 23) and in one of two LLJ hybrids from the Sawmill River drainage (Site 26). A tetraploid LLJJ, also from Site 23, was a Got-1<sup>110/110/100/79</sup> heterozygote. It would appear that the tetraploid was derived from a Got-1<sup>100</sup>-bearing LLJ hybrid or that sperm was incorporated from bisexual individuals in the population that possessed the allele. Unfortunately, no bisexual individuals were collected from Site 23. Pgm-2<sup>100</sup> is mostly monomorphic in all genomic combinations of hybrids and in the bisexual species, but there are two rare alleles at this locus. Pgm-2<sup>120</sup> was found in a single LLJ hybrid (Pgm-2<sup>120/120/100</sup>) from Site 3. An allele with the same mobility has been found in *A. laterale* from northwestern Ontario and in *A. laterale* and LLJ hybrids in central Ontario (Lowcock and Bogart, 1989). Lowcock and Bogart suggested that Pgm-2<sup>120</sup> provided evidence for either an eastern and southern dispersion across the arc of limestone islands extending from the Michigan Upper Peninsula to the Bruce Peninsula in Lake Huron or a post-Wisconsin northern and eastern dispersion around Lakes Huron, Michigan, and Superior from a southwestern relictual population. Finding the allele in eastern New York (Site 3) but not in more western sites (such as southern Ontario) does not support their hypothesis of a northeastern dispersion. Pgm-2<sup>75</sup> was found as a rare allele in one heterozygous *A. jeffersonianum* from the top of Snake Mountain, Addison County, Vermont (Site 76), an LJJ hybrid from High Woods, Ulster County, New York (Site 5); and two LLJ hybrids, one from the base of Snake Mountain, Addison County, Vermont (Site 77), and the other from Hazen Point Marsh, Grand Isle County, Vermont (Site 88). If this allele is a *jeffersonianum* marker that relates these four sites and two triploid hybrid genotypes, the Vermont LLJ hybrids might have been derived from LJJ hybrids, and the LJJ hybrids on the west side of the Hudson

←

are LLJ (1, 3, 8, 9, 11, 12, 14, and 15), LLJ (samples 2 and 6), and LJ (sample 4). SOD is also a dimeric enzyme and demonstrates good separation of the diagnostic alleles. *Ambystoma laterale* bands (samples 1, 3–6, and 8–10) migrate much farther from the origin than do *A. jeffersonianum* bands (samples 2 and 15). The hybrids on the SOD gel are LLJ (sample 7) and LJJ (samples 12–14 and 16).

TABLE 4  
Allele Frequencies for Diploid and Polyploid Salamanders

Locus	<i>n</i>	Genotype ( <i>n</i> ) <sup>a</sup>								Mobility <sup>b</sup>
		JJ (66)	IL (231)	LJ (84)	LJJ (256)	LLJ (319)	LJJJ (15)	LLJJ (6)	LLLJ (25)	
Adh	<i>n</i>	(66)	(129)	(58)	(148)	(218)	(7)	(3)	(6)	
	A	0.955	0.426	0.672	0.806	0.840	0.964	0.917	0.859	+375
	C	0.045	0.574	0.328	0.194	0.160	0.036	0.083	0.141	+100
AcPh-2	<i>n</i>	(55)	(157)	(70)	(190)	(247)	(11)	(3)	(22)	
	A	0.109	0.946	0.607	0.531	0.727	0.568	0.500	0.602	-100
	B	0.800	—	0.264	0.453	0.252	0.409	0.333	0.398	-83
	C	0.091	0.054	0.129	0.016	0.021	0.023	0.167	—	-59
Acon-1	<i>n</i>	(54)	(142)	(64)	(165)	(159)	(10)	(2)	(11)	
	A	0.139	0.958	0.633	0.406	0.600	0.350	0.125	0.636	+100
	B	0.861	0.042	0.367	0.354	0.400	0.650	0.825	0.364	+79
Ck-1	<i>n</i>	(51)	(224)	(68)	(136)	(301)	(15)	(2)	(20)	
	A	1.000	1.000	0.927	0.931	1.000	1.000	1.000	1.000	+100
	B	—	—	0.073	0.069	—	—	—	—	+80
Ck-2	<i>n</i>	(63)	(224)	(76)	(147)	(301)	(10)	(2)	(20)	
	A	—	1.000	0.507	0.426	0.648	0.325	0.625	0.675	-100
	B	1.000	—	0.493	0.574	0.352	0.675	0.375	0.325	-380
Est-1	<i>n</i>	(15)	(150)	(23)	(67)	(80)	(1)	(1)	(3)	
	A	—	1.000	0.522	0.363	0.684	0.250	0.500	0.833	+100
	B	1.000	—	0.478	0.637	0.316	0.750	0.500	0.167	+80
Got-1	<i>n</i>	(66)	(231)	(84)	(254)	(316)	(15)	(6)	(25)	
	A	—	—	—	—	0.007	—	0.083	—	+110
	B	—	1.000	0.500	0.337	0.659	0.250	0.417	0.760	+100
	D	1.000	—	0.500	0.663	0.334	0.750	0.500	0.240	+79
Got-2	<i>n</i>	(66)	(229)	(79)	(243)	(319)	(15)	(6)	(25)	
	A	1.000	—	0.494	0.654	0.359	0.733	0.542	0.260	-180
	B	—	0.998	0.506	0.346	0.641	0.267	0.458	0.740	-100
	C	—	0.002	—	—	—	—	—	—	-50
Idh-1	<i>n</i>	(66)	(224)	(79)	(229)	(318)	(15)	(6)	(25)	
	A	1.000	—	0.500	0.656	0.329	0.717	0.542	0.280	+142
	B	—	1.000	0.500	0.344	0.671	0.283	0.458	0.720	+100
Ldh-1	<i>n</i>	(66)	(231)	(84)	(243)	(319)	(15)	(6)	(25)	
	A	0.008	—	—	—	—	—	—	—	+115
	B	—	0.993	0.530	0.365	0.693	0.267	0.625	0.770	+100
	C	0.992	0.007	0.470	0.635	0.307	0.733	0.375	0.230	+88
Ldh-2	<i>n</i>	(66)	(231)	(80)	(245)	(302)	(15)	(6)	(25)	
	A	—	0.816	0.400	0.063	0.367	0.050	0.208	0.360	+130
	B	1.000	0.184	0.594	0.937	0.633	0.950	0.792	0.640	+100
	C	—	—	0.006	—	—	—	—	—	+55
Mdh-1	<i>n</i>	(65)	(231)	(84)	(252)	(318)	(15)	(6)	(25)	
	B	0.985	—	0.500	0.667	0.378	0.750	0.500	0.270	+176
	C	—	—	—	—	0.005	—	—	—	+135
	D	0.015	1.000	0.500	0.333	0.617	0.250	0.500	0.730	+100
Mdh-2	<i>n</i>	(66)	(231)	(84)	(255)	(318)	(15)	(6)	(25)	
	A	—	—	—	0.003	—	—	—	—	+125
	B	0.962	0.989	0.982	0.983	0.996	1.000	1.000	0.980	+100
	C	0.038	0.011	0.018	0.014	0.004	—	—	0.020	+34

TABLE 4  
(Continued)

Locus	Genotype ( <i>n</i> ) <sup>a</sup>								Mobility <sup>b</sup>
	JJ (66)	IL (231)	LJ (84)	LJJ (256)	LLJ (319)	LJJJ (15)	LLJJ (6)	LLLJ (25)	
Mdh-3	<i>n</i> (66)	(231)	(84)	(255)	(319)	(15)	(6)	(25)	
	A	—	—	—	0.002	—	0.083	0.010	-160
	B	1.000	1.000	1.000	0.998	1.000	0.917	0.990	-100
Me-1	<i>n</i> (52)	(178)	(72)	(202)	(249)	(11)	(2)	(15)	
	A	0.327	0.953	0.479	0.555	0.537	0.523	0.250	0.700
	B	0.644	0.047	0.521	0.443	0.463	0.477	0.750	0.300
	C	0.029	—	—	0.002	—	—	—	+60
Mpi	<i>n</i> (31)	(115)	(53)	(86)	(147)	(9)	(2)	(12)	
	A	1.000	0.096	0.179	0.667	0.383	0.750	0.375	0.250
	B	—	0.904	0.500	0.333	0.617	0.250	0.625	0.750
	C	—	—	0.321	—	—	—	—	+80
6Pgd	<i>n</i> (58)	(185)	(58)	(200)	(240)	(6)	(2)	(2)	
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	+100
Pgi	<i>n</i> (32)	(141)	(36)	(128)	(205)	(9)	(1)	(16)	
	A	0.812	0.128	0.556	0.623	0.392	0.667	0.750	0.381
	B	0.188	0.039	0.125	0.093	0.024	0.111	—	0.032
	C	—	0.833	0.319	0.283	0.584	0.222	0.250	0.587
Pgm-1	<i>n</i> (53)	(213)	(72)	(230)	(251)	(14)	(5)	(22)	
	B	0.793	1.000	0.806	0.864	0.856	0.804	0.900	0.955
	C	0.198	—	0.194	0.136	0.144	0.196	0.100	0.045
	D	0.009	—	—	—	—	—	—	+82
Pgm-2	<i>n</i> (66)	(231)	(84)	(256)	(319)	(15)	(6)	(25)	
	A	—	—	—	0.002	—	—	—	+120
	B	0.992	1.000	1.000	0.999	0.996	1.000	1.000	1.000
	C	0.008	—	—	0.001	0.002	—	—	+75
Sod-1	<i>n</i> (66)	(231)	(84)	(253)	(316)	(14)	(6)	(25)	
	B	—	1.000	0.500	0.333	0.633	0.250	0.500	0.740
	D	1.000	—	0.500	0.667	0.367	0.750	0.500	0.260

<sup>a</sup> Sample sizes in parentheses are included for the numbers of individuals of each genome that were used in the study as well as the number that were examined and scored for each locus.

<sup>b</sup> Mobilities are the relative mobilities of the alleles on the gel compared with the most common allele found in *A. laterale*. Positive mobilities (+) indicate migration from the origin toward the anode and negative mobilities (-) indicate migration toward the cathode.

River are affiliated with Vermont *A. jeffersonianum*.

#### DISTRIBUTION OF GENOMIC CLASSES

The distribution of *A. jeffersonianum* and *A. laterale* in Connecticut and adjacent states was described by Klemens (1993) and included sites sampled in this investigation. Klemens also outlined the types of habitat characteristic of these species. *Ambystoma laterale* typically breeds in floodplain marsh-

es and wooded swamps and can tolerate continuous, slow water movement, whereas *A. jeffersonianum* is found in discrete vernal pools with little or no water movement and usually located in rocky, moderately to steeply graded, forested areas. *Ambystoma jeffersonianum* has a more western distribution. In New York, we found *A. jeffersonianum* in the Finger Lakes region and on both sides of the Hudson River as far north as Albany. We found this species west of the Connecticut River, in southern Vermont, Massachusetts,

TABLE 5  
Hybrid Individuals that Demonstrated a Homozygous Condition for the Diagnostic Alleles

Locus	Genotype ( <i>n</i> ) <sup>a</sup>						Total	%
	LJ	LJJ	LLJ	LJJJ	LLJJ	LLLJ		
Ck-2	<i>n</i> A B	(76) — —	(147) — —	(301) 8 2	(10) — —	(2) — —	(20) — —	(556) 9 2
								1.6 0.4
Got-1	<i>n</i> B D	(84) — —	(254) — —	(316) 2 —	(15) — —	(7) — —	(25) 1 —	(701) 3 0
								0.4 0.0
Got-2	<i>n</i> A B	(79) — 1	(243) — 1	(319) — 1	(15) — —	(6) — —	(25) — —	(687) 0 3
								0.0 0.4
Idh-1	<i>n</i> A B	(79) 2 2	(229) 7 2	(318) 2 15	(15) — —	(6) — —	(25) 1 1	(672) 12 20
								1.8 3.0
Ldh-1	<i>n</i> B C	(84) 5 —	(243) 2 3	(319) 35 1	(15) — —	(6) 5 —	(25) 4 —	(692) 52 4
								7.5 0.6
Mdh-1	<i>n</i> B D	(84) — —	(252) 1 —	(318) — —	(15) — —	(6) — —	(25) — —	(700) 1 0
								0.1 0.0
Mpi	<i>n</i> A B	(53) — —	(86) — —	(147) 9 2	(9) — —	(2) — 1	(12) — —	(309) 9 3
								2.9 0.9
Sod-1	<i>n</i> B D	(84) — —	(253) — —	(316) — 4	(14) — —	(6) — —	(25) — —	(698) 0 4
								0.0 0.6

<sup>a</sup> Numbers in parentheses are the number of hybrid individuals of each genomic group examined for each locus and the total number of hybrids examined from all 106 sites.

and Connecticut. *Ambystoma jeffersonianum* was also found at Snake Mountain in northwestern Vermont (Site 76). French and Master (1988) collected a specimen at Winchester in extreme southwestern New Hampshire (Site 73), slightly east of the Connecticut River, which is the most eastern locality for *A. jeffersonianum* and the only New Hampshire locality for the species. We collected three LJJ hybrids near the northern edge of Quabbin Reservoir (Site 63) in Franklin County just west of the Worcester County line in Massachusetts. Assuming that *A. jeffersonianum* is the expected sperm donor for these hybrids, Site 63 would represent the easternmost locality for this species in New England.

*Ambystoma laterale* is widely distributed from western New York northeastward

across southern New England into Maine. It is extremely uncommon in southeastern New England. In this area, *A. laterale* was found to occur at a few scattered sites without associated hybrids. We did not find this species in Rhode Island despite intensive searches (C. Raithel personal commun.).

Because the hybrids outnumbered the bisexual species at most sites, the distribution of the bisexual species was often inferred from the expectation that *A. jeffersonianum* was present at sites inhabited by LJL hybrids and that *A. laterale* occurred with LLJ hybrids. At sites where bisexual species and hybrids were found together, the relationship was realized: LLJ and LLLJ were found with *A. laterale*; LJJ and LJJJ were found with *A. jeffersonianum*.

Diploid (LJ) hybrids were found in asso-

TABLE 6  
Reversed Genotypes Found in LJJ Triploid Hybrids

Site ( <i>n</i> ) <sup>a</sup>	Diagnostic locus ( <i>n</i> ) <sup>b</sup>							
	Ck-2 (147)	Got-1 (254)	Got-2 (243)	Idh-1 (229)	Ldh-1 (243)	Mdh-1 (252)	Mpi (86)	Sod-1 (253)
6 (14)	1	—	—	—	2	—	—	—
11 (9)	2	—	—	—	1	—	1	—
12 (16)	—	—	—	—	1	—	—	—
13 (7)	3	1	1	1	1	—	1	—
14 (5)	1	—	—	—	1	—	1	—
19 (7)	7	—	—	5	6	—	—	—
20 (8)	2	—	—	—	—	—	—	—
22 (7)	6	—	—	—	—	—	—	—
23 (1)	—	1	—	—	—	—	—	—
30 (6)	5	—	—	—	—	—	—	—
32 (9)	1	1	1	1	1	—	—	—
33 (1)	1	—	—	—	—	1	—	—
34 (3)	—	—	—	—	1	—	—	—
41 (7)	—	—	—	—	1	—	—	—
43 (11)	1	—	—	1	5	—	—	—
46 (26)	2	—	—	—	—	—	—	—
51 (23)	3	—	—	—	1	—	—	—
72 (1)	1	—	—	—	—	—	—	—
76 (13)	4	—	5	2	1	—	—	—
Total	40	3	7	10	22	1	3	0
% LJJ <sup>c</sup>	27.2	1.2	2.9	4.4	9.0	0.4	3.5	0.0

<sup>a</sup> Unexpected reversals were found in individuals from 19 of 106 sites (appendix A). The number of LJJ individuals at each site (*n*) may be fewer than the number of reversed individuals because some specimens demonstrated more than one reversal.

<sup>b</sup> Diagnostic loci are essentially fixed in the pure species and are used to identify the genomic contents in hybrids. The sample size represents the total number of LJJ individuals examined.

<sup>c</sup> The percentage (%) is the percentage of the LJJ individuals analyzed for each locus that had a reversed genotype.

ciation with either *A. laterale* or *A. jeffersonianum* and always were found with triploid hybrids. At some sites, the number of diploid hybrids was greater than the number of triploid hybrids; Site 3, with 21 diploid and 13 triploid hybrids, had the most diploid hybrids of any site. Some diploid hybrids demonstrated homozygous genotypes for a few diagnostic alleles, but heterozygotes for these same alleles were found in other diploid hybrids from the same site. The diploid hybrids did not express any rare or unique alleles. These data suggest that the diploid hybrids do not form independent lineages and could not be construed as the original hypothetical hybrid that may have given rise to hybrids of higher ploidy levels. The presence, evolution, and mode of reproduction of the diploid hybrids is problematic. It was suggested

by Bogart (1989) that the diploid hybrids (1) could have been produced in situ from LLJ, LJJ, or tetraploid hybrids through meiotic reduction or (2) are the result of the continual creation of first-generation hybrids independently in several populations by hybridization of *A. laterale* and *A. jeffersonianum*. The second hypothesis was tested by Hedges et al. (1992) by sequence analyses of a portion of the cytochrome *b* gene from mtDNA derived from diploid hybrids and from sympatric *A. jeffersonianum* or *A. laterale* used in the present investigation. Because mtDNA is inherited from the female, a recent hybrid would be expected to have either an *A. laterale* or an *A. jeffersonianum* sequence. Because none of the examined hybrids, including the diploid hybrids, had a *laterale* or *jeffersonianum* sequence, the recent hybridiza-

TABLE 7  
Reversed Genotypes Found in LLJ Triploid Hybrids

Site ( <i>n</i> ) <sup>a</sup>	Diagnostic locus ( <i>n</i> ) <sup>b</sup>							
	Ck-2 (301)	Got-1 (316)	Got-2 (319)	Idh-1 (318)	Ldh-1 (319)	Mdh-1 (318)	Mpi (147)	Sod-1 (316)
17 (6)	—	—	—	—	1	—	—	—
18 (17)	2	1	—	1	—	3	4	1
23 (13)	—	—	—	2	—	12	—	—
26 (2)	—	—	—	—	—	1	—	—
36 (24)	—	—	—	—	1	—	—	—
40 (15)	—	—	—	—	—	—	1	—
42 (12)	—	—	—	—	—	6	—	—
45 (18)	—	—	—	—	—	6	—	—
48 (2)	—	—	—	—	—	1	—	—
50 (6)	2	—	—	—	2	—	—	—
59 (1)	—	—	—	—	—	1	—	—
62 (15)	2	—	—	1	1	4	—	—
67 (8)	2	—	1	—	—	—	—	—
70 (4)	1	—	—	1	—	—	—	—
74 (2)	—	—	—	—	—	—	1	—
75 (12)	1	—	—	—	—	4	—	—
77 (5)	—	—	1	1	—	—	—	—
80 (11)	2	—	—	—	—	—	—	—
82 (5)	3	—	—	—	—	—	—	—
84 (6)	—	—	2	—	—	—	—	3
85 (1)	—	—	1	—	—	—	—	—
87 (10)	5	—	1	—	—	3	—	2
88 (7)	—	—	3	—	—	—	—	3
89 (24)	—	—	17	1	1	—	—	15
95 (10)	—	—	—	—	2	2	—	—
103 (1)	—	—	—	—	—	—	—	1
Total	20	1	26	7	8	43	6	25
% LLJ <sup>c</sup>	6.6	0.3	8.2	2.2	2.5	13.5	4.1	7.9

<sup>a</sup> Unexpected reversals were found in individuals from 26 of 106 sites (appendix A). The number of LLJ individuals at each site (*n*) may be fewer than the number of reversed individuals because some specimens demonstrated more than one reversal.

<sup>b</sup> Diagnostic loci are essentially fixed in the pure species and are used to identify the genomic contents in hybrids. The sample size represents the total number of LLJ individuals examined.

<sup>c</sup> The percentage (%) is the percentage of the LLJ individuals analyzed for each locus that had a reversed genotype.

tion of these two species cannot be considered a viable hypothesis. The diploid hybrids must be produced independently from the other hybrids. The only information on the reproductive biology of diploid hybrids within this complex comes from a study of *A. laterale-texanum* hybrids on Pelee (Bogart et al., 1985) and Kelleys (Bogart et al., 1987) Islands in Lake Erie. These diploid hybrids produced both diploid and triploid hybrids, but diploid hybrids were not produced by any triploid or tetraploid hybrids that were examined.

Tetraploid hybrids were not commonly found, and none of the sites could be classified as "pure" tetraploid sites. The low frequency of tetraploids found at a variety of sites is in keeping with experimental information (Bogart et al., 1989) demonstrating that ploidy elevation from triploid to tetraploid is fairly common and is influenced by temperature. Bogart et al. found that more tetraploids were produced through sperm incorporation in unreduced eggs when the temperature was 15°C than when the temperature was 6°C, but some tetraploids were produced

TABLE 8  
Electrophoretic Analysis of 19 Progeny from a Site 89<sup>a</sup> Female that Possessed Reversed Genotypes<sup>b</sup>  
for Four Polymorphic Loci

Specimen, AMNH no.	Sex <sup>c</sup>	Ploidy	Locus			
			Got-2	Idh-1	Ldh-1	Sod-1
<b>Female</b>						
139498	F	3n	(AAB)	(AAB)	BBC	(BDD)
<b>Triploid offspring</b>						
1) 140449	L	3n	(AAB)	ABB	BBC	BBD
2) 140450	L	3n	(AAB)	ABB	BBC	(BDD)
3) 140451	L	3n	ABB	ABB	BBC	BBD
4) 140458	F	3n	(AAB)	ABB	BBC	(BDD)
5) 140452	L	3n	(AAB)	ABB	BBC	BBD
6) 140460	F	3n	ABB	ABB	(BCC)	BBD
7) 140453	L	3n	(AAB)	ABB	BBC	BBD
8) 140454	L	3n	ABB	ABB	BBC	BBD
9) 140455	L	3n	(AAB)	ABB	BBC	(BDD)
10) 140465	F	3n	ABB	ABB	BBC	BBD
11) 140466	F	3n	(AAB)	ABB	BBC	BBD
<b>Tetraploid offspring</b>						
12) 140457	F	4n	ABBB	ABBB	BBBC	(BBDD)
13) 140459	F	4n	ABBB	ABBB	BBBC	(BBDD)
14) 140461	F	4n	ABBB	ABBB	BBBC	(BBDD)
15) 140462	F	4n	(AABB)	ABBB	BBBC	(BBDD)
16) 140463	F	4n	(AABB)	ABBB	BBBC	(BBDD)
17) 140464	F	4n	ABBB	ABBB	BBBC	(BBDD)
18) 140467	F	4n	ABBB	ABBB	BBBC	BBBB
19) 140456	L	4n	ABBB	ABBB	BBBC	BBBB

<sup>a</sup> See appendix A for site and specimen information.

<sup>b</sup> A reversed genotype (in parentheses) for a diagnostic locus would have an LJJ electrophoretic phenotype in an LLJ hybrid.

<sup>c</sup> Some of the offspring were sampled as larvae (L) and the sex could not be differentiated.

at the lower temperature as well. Lowcock et al. (1991) found a greater than expected number of tetraploid LLLJ individuals in a central Ontario population, and Lowcock and Murphy (1991) reported a pentaploid LLLLJ individual. These data show that tetraploids are normally encountered in hybrid populations and are derived from triploids. It appears that tetraploids are not as viable as triploids, which would explain the low frequency of their occurrence and the fact that no pure tetraploid populations have been found. Tetraploid hybrids were found to produce triploid and tetraploid hybrid offspring on Pelee Island (Bogart and Licht, 1986), but Pelee Island hybrids do not have a *jeffersonianum* genome. The rate of tetraploid production and the ploidy of any progeny that

may be produced by New York and New England tetraploid hybrids are not known.

#### EVOLUTIONARY SIGNIFICANCE OF HYBRIDS

The *Ambystoma* hybrids in New York and New England form part of a much wider ranging phenomenon that includes "nuclear" hybrids of four distinct species. *Ambystoma texanum* does not range into the geographical area examined in this study, and no hybrids were found with that genome. Hybrids involving *A. tigrinum* also were not found, although this species is sympatric with *A. laterale* on Long Island. Neither *A. maculatum* nor *A. opacum*, which are wide ranging and sympatric with the New York and New England hybrids, have ever been found to be

involved in the formation of hybrids. It is expected that a genomic contribution of *A. maculatum* is lethal (Bogart et al., 1989; Taylor, 1992) and that genomic contribution from the autumn-and-terrestrial-breeding *A. opacum* is not possible. The hybrids in New York and New England possess combinations of only two of the four species, *A. laterale* and *A. jeffersonianum*.

Based on the current information, it is possible to construct a hypothetical paradigm that outlines the evolution and evolutionary trajectory of these hybrids. At the outset, it must be realized that these hybrids do not fit models that have been proposed for other breeding systems (Maynard-Smith, 1978; Bell, 1982; Dawley and Bogart, 1989), which renders comparisons to other organisms difficult.

A distinction must be made between the evolution of the cytoplasm and that of the nucleus. If the information obtained from cytochrome *b* sequences (Hedges et al., 1992) can be applied to the cytoplasmic contents generally, then all of the hybrids possess cytoplasm that is descended from some very early ancestor that lived millions of years ago. The genomic content of the nucleus in this very early form is not known and may not even have been a hybrid, but the early ancestral form must have had some form of all-female reproduction to perpetuate the "hybrid" cytoplasm. Reproduction could have been by parthenogenesis, gynogenesis, or hybridogenesis, because all of these mechanisms would conserve the female cytoplasm; however, some form of hybridogenesis that enabled sperm to be incorporated would be the only mechanism that would allow the nuclear contents to evolve and produce the variety of hybrids observed at the present time.

A hypothetical ancestor with a nuclear genome of XX could, through incorporation of sperm from *A. laterale*, produce XL females or XXL females. As well as the obvious advantage of incorporating adaptively significant genes from the sympatric bisexual male, the new XL or XXL hybrid would be expected to have an advantage over XX female salamanders in her competitive ability to attract *A. laterale* males and to obtain necessary spermatophores. It might be difficult for

males to discriminate between the hybrid and bisexual females if the female hybrids contained normal genes derived from males in sympatry. Over time, and especially in association with other potential sperm donors, the X genomes would be completely replaced with a viable combination of genomes determined by the sympatric associations the hybrids encountered through time. In New York and New England, the combinations are mostly LLJ and LJJ, but if *A. texanum* (TT) was sympatric with an XX or an XXL hybrid, the descendants would be LLT or LTT, which are common genotypes on Pelee Island and Kelleys Island in Lake Erie. Gynogenetic or parthenogenetic reproduction could have a short-term advantage in increasing the number of homogeneous individuals, but range extensions and the long-term survival of hybrids appear to be facilitated by frequent nuclear genome replacement in hybrid lineages. Genome replacement is the mechanism that best explains the great diversity of "nuclear" hybrids that maintain contemporary genomes in cytoplasm that is unlike that of any of the contemporary species.

More than half a century ago, Clanton (1934) hypothesized that the fate of mixed bisexual and unisexual hybrid populations would be extinction because the proportion of males would diminish to subcritical levels. So far, there are no documented cases of populations of hybrids that have been extirpated as a direct result of "the Clanton Effect," as coined by Minton in 1954. *Ambystoma jeffersonianum* and its associated triploid hybrid, LJJ, appear to be found in less disturbed, upland localities (see Klemens, 1990, 1993), so they may be more vulnerable to habitat disturbance than is *A. laterale* and its associated triploid hybrid, LLJ. Apart from the Clanton Effect, there appear to be two basic pathways leading to the extirpation of JJ/LJJ. The first pathway is "classical" extinction, where habitat disturbance and alteration render the site unsuitable for *A. jeffersonianum* and the species dies out. Without male *A. jeffersonianum*, we would expect the concomitant extirpation of LJJ but, at sites where *A. jeffersonianum* and *A. laterale* are generally sympatric, extinction of LJJ probably proceeds along a second pathway. Hy-

brid LJJ can reproduce by using the sperm of *A. laterale* and, through gynogenesis, LJJ could persist in the absence of JJ until, by the process of genome replacement, the hybrids are converted to LLJ. The rate of conversion is determined by the frequency of sperm incorporation in unreduced eggs and natural selection, especially among the offspring. Judging by the fact that mixed LJJ/LLJ populations exist (three sites in the present study and a few sites in southern Ontario [Bogart, 1982]), but are very uncommon, the rate might be rapid. It is also possible that the initial stages of sperm incorporation could stimulate the production of tetraploids. We found the presence of the rarer hybrid genotypes, i.e., LLLJ, LLJJ, and LJJJ, within populations to be positively correlated with close proximity of the two bisexual species. This is the current situation at Snake Mountain, Addison County, Vermont, where large, robust populations of JJ and LJJ occur on the wooded ridge and use discrete vernal pools for breeding. Dense populations of LL and LLJ occur in the extensive, interconnected marshes and swamps at the base of the ridge. If the ridge-top habitat became less suitable for JJ/LJJ, most likely through habitat disturbance, invasion of LL into that population would be predictable and would result in a shift from JJ/LJJ to LL/LLJ. Because *A. laterale* tolerates a broader range of habitats and exists in more disturbed environments than does *A. jeffersonianum* (Klemens, 1990, 1993), the conversion of an LL/LLJ population to a JJ/LJJ population through the dispersion of *A. jeffersonianum* would be a less common event.

All of the hybrid genomic groups demonstrate homozygosities and reversals that are consistent with the recombination events predicted by Asher and Nace (1971). Thus, all of the diploid, triploid, and tetraploid hybrids are expected to undergo a doubling of the chromosomes to respective tetraploid, hexaploid, and octoploid levels prior to meiotic diplotene. Such doubling is likely an important facet of the hybrid's reproduction and could be a rapid method of restructuring genomic groupings. For example, the hypothetical triploid XXL hybrid would engage in duplication, so the oocyte would be expected to be XXXXLL; through homologous

(XX homologous chromosomes) or homologous (XL "homologous" chromosomes) pairing, this hybrid could produce a variety of "unreduced" triploid eggs that could include XLL through purely asexual or gynogenetic automictic reproduction. This possibility was explored more completely by Taylor (1991), who analyzed chromosome markers and electrophoretic phenotypes in LJJ females and their offspring.

## CONCLUSIONS

When we began our investigation, it was widely assumed that the polyploid "hybrid species" were much rarer than the bisexual species. We now know the reverse to be true. Bisexual individuals usually exist at low levels in populations composed mostly of hybrids. We discovered only a few populations consisting solely of bisexual individuals. These were all *A. laterale*. The populations of *A. laterale* that occur on the eastern end of Long Island (Site 71), in extreme eastern Connecticut (Sites 57 and 60), and in southeastern Massachusetts (Site 61 and possibly Site 66) are unique because they do not contain hybrids. These bisexual populations most likely represent an old lineage of *A. laterale* and were either not in contact with an ancestral hybrid progenitor (possibly through geographic isolation) or competitively excluded the hybrids. Such populations are significant because they provide comparative data for *A. laterale* subjected to the normal processes of population genetics, evolving through time unencumbered by hybrid competition.

We did not find any sizable populations of *A. jeffersonianum*, which is disturbing because this species is probably much less common than its published range would suggest. Conant and Collins (1991) mapped an extensive range for *A. jeffersonianum* in eastern North America; however, there are no karyological and electrophoretic data for the majority of the range. Data from mainland Ohio (Selander et al. 1993; Selander, 1994) and Indiana (Morris, 1985; Morris and Brandon, 1984) demonstrate that hybrids with J and L genomes have incorporated *A. texanum* and *A. tigrinum* in addition to *A. laterale*. These hybrids confound the actual distribu-

tion and identification of "pure" *A. jeffersonianum*. It is an interesting facet of the complex that *A. jeffersonianum* genes and genomes are common and widely spread in eastern North America, but they mostly reside in hybrids.

In our attempt to outline the ranges of two species of *Ambystoma* and their hybrids, we have uncovered a unique genetic and evolutionary system that warrants additional study. The hybrid's genetic system appears complex because there is no precedence for such a system among other vertebrates. The system

in New York and New England, which involves only two bisexual species, will probably prove to be simple compared with systems where hybrids interact with three or more species. The recognition of the species and hybrids over the entire range would seem to be an important priority, to conserve and to protect species and hybrid combinations that could be essential for improving our understanding of the complex. It will also be necessary to monitor different hybrid combinations over time to document the interactions that lead to genome replacement.

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## APPENDIX A

Localities where *Ambystoma laterale* (LL), *A. jeffersonianum* (JJ), and their diploid (LJ), triploid (LLJ or LJL), and tetraploid (LLLJ, LLJJ, LJJJ) hybrids used in this study were collected are listed by site number (figures 1, 2; table 2). Catalog numbers refer to specimens deposited in the American Museum of Natural History (AMNH), University of Michigan Museum of Zoology (UMMZ), or the Museum of Comparative Zoology (MCZ).

### NEW YORK, CONNECTICUT, AND MASSACHUSETTS ALLEGHENY RIVER DRAINAGE

1. New York: Cattaraugus County: Olean, Camel Back. LL: (AMNH 129233–35). LJ: (AMNH 129236). LLJ: (AMNH 129237).

### FINGER LAKES DRAINAGE

2. New York: Tompkins County: Dryden. JJ: (AMNH 122156–58).

### HUDSON RIVER DRAINAGE (WEST BANK)

3. New York: Albany County: Swamps below Helderberg Escarpment. LL: Guilderland (AMNH 129238–40); New Scotland (AMNH 129241). LJ: Guilderland (AMNH 129242–54); New Scotland (AMNH 129255–62). LLJ: Guilderland (AMNH 129267–75); New Scotland (AMNH 129263–66). LLLJ: Guilderland (AMNH 129276).
4. New York: Albany County: Coeymans. LJ: (AMNH 129277).
5. New York: Ulster County: High Woods. JJ: (AMNH 122134–35). LJ: (AMNH 122136–55).
6. New York: Ulster County: West Park. JJ: (AMNH 122119). LJ: (AMNH 122120–33).
7. New York: Orange County: 2 mi SE of Chester, Seely Brook Drainage. LL: (AMNH 129278–80). LJ: (AMNH 129281–84). LLJ: (AMNH 129285–86). LLLJ: (AMNH 129287).

### HUDSON RIVER DRAINAGE (EAST BANK)

8. New York: Columbia County: Chatham. LJ: (AMNH 122087).
9. New York: Columbia County: Ancram. LJ: (AMNH 139537).
10. New York: Dutchess County: Red Hook. LJ: (AMNH 122088–89).
11. New York: Dutchess County: Hyde Park, Val-kill. LJ: (AMNH 131560–68). LLJ: (AMNH 131569). LJ: (AMNH 129288–89).
12. New York: Dutchess County: Clinton Hollow. LJ: Jug Hill Camp (AMNH 122104, AMNH 122114). LJ: Jug Hill Camp (AMNH 122105–09, AMNH 122111–12, AMNH 122115–16); between Clinton Hollow and Jug Hill Camp (AMNH 122097–103). LJ: Jug Hill Camp (AMNH 122110, AMNH 122113).
13. New York: Dutchess County: Stanford, 0.5 mi S Ryder Pond. JJ: (AMNH 131570–76). LJ: (AMNH 131577–83, AMNH 131591). LJ: (AMNH 131584–90).
14. New York: Dutchess County: Stanford, Tamarack Swamp. JJ: (AMNH 131592–93). LJ: (AMNH 131594–98).
15. New York: Dutchess County: Stanford, 0.3 mi N Ryder Pond. LLJ: (AMNH 131600). LJ: (AMNH 131599).
16. New York: Dutchess County: Freedom Plains. LLJ: (AMNH 122117–18).
17. New York: Dutchess County: Wappinger. LL: (AMNH 131601–04). LJ: (AMNH 131605–07). LLJ: (AMNH 131608–13). LLLJ: (AMNH 131614).
18. New York: Dutchess County: Pawling, Great Swamp. LL: (AMNH 139538, AMNH 145056). LJ: (AMNH 145036, AMNH 145042, AMNH 145048–49, AMNH 145051–52, AMNH 145054, AMNH 145057, AMNH 145060–61). LLJ: (AMNH 135600, AMNH 140443–44, AMNH 145035, AMNH 145037–41, AMNH 145043–46, AMNH 145050, AMNH 145053, AMNH 145055, AMNH 145059). LLLJ: (AMNH 145047).
19. New York: Dutchess County: Pawling, Quaker Ridge. LJ: (AMNH 122090–96).
20. New York: Putnam County: Putnam Valley. JJ: (AMNH 134461). LJ: (AMNH 129301–03). LJ: (AMNH 129304–10, AMNH 134462).
21. New York: Westchester County: Glendale. LJ: (AMNH 135601).
22. New York: Columbia and Dutchess Counties, and Massachusetts: Berkshire County: Taconic Escarpment, western versant. JJ:

Dutchess County: Northeast (AMNH 129290). LJ: Columbia County: Hillsdale (AMNH 129293–95); Dutchess County: Northeast (AMNH 129291). LJJ: Columbia County: Hillsdale (AMNH 129296–300); Dutchess County: Northeast (AMNH 129292); Berkshire County: Mount Washington (AMNH 129314).

23. Connecticut: Fairfield County: Corner Pond Brook drainage. LJ: Danbury (AMNH 121985–86, AMNH 121990, AMNH 121992–97). LLJ: Danbury (AMNH 121977–84, AMNH 121987, AMNH 121989); New Fairfield (AMNH 121974–76). LJJ: Danbury (AMNH 121991). LLJJ: Danbury (AMNH 121988).

24. New York: Putnam County: Bog Brook drainage. LL: Southeast (AMNH 135598–99).

**HOUSATONIC RIVER DRAINAGE (WEST BANK)**

25. Connecticut: Fairfield County: New Fairfield. LJJ: (AMNH 114070).

26. Connecticut: Fairfield County: Sawmill River drainage. LJ: (AMNH 121973). LLJ: (AMNH 121971–72).

27. Connecticut: Fairfield County: Bethel. JJ: (AMNH 114051–52). LJJ: (AMNH 114059–64, AMNH 121968–70, AMNH 129412).

28. Connecticut: Fairfield County: Redding. LJJ: (AMNH 114065).

29. Massachusetts: Berkshire County: Lenox. LJJ: (AMNH 114080).

30. Massachusetts: Berkshire County: West Stockbridge. JJ: (AMNH 129316). LJ: (AMNH 129317–21). LJJ: (AMNH 129322–27).

31. Connecticut: Litchfield County and Massachusetts: Berkshire County. Taconic Escarpment, eastern versant. JJ: Berkshire County: Egremont (AMNH 131615). LJJ: Berkshire County: Sheffield (AMNH 129315); Litchfield County: Joyceville (AMNH 134463–64); Salisbury (AMNH 134484).

32. Massachusetts: Berkshire County: North Egremont. JJ: (AMNH 131616–19). LJJ: (AMNH 131620–28). LJJJ: (AMNH 131629).

33. Connecticut: Litchfield County: Lime Rock. LJJ: (AMNH 122009).

34. Connecticut: Litchfield County: Sharon. JJ: (AMNH 114054). LJJ: (AMNH 114066, AMNH 129419–20).

35. Connecticut: Litchfield County: Kent. JJ: (AMNH 129413–17). LJ: (AMNH 129418).

36. Connecticut: Litchfield County: Salisbury, Washining Lake. LL: (AMNH 134465, AMNH 139375). LLJ: (AMNH 134466–81, AMNH 135603, AMNH 139376–82).

37. Connecticut: Litchfield County: Taconic. LJJ: (AMNH 134482).

38. Connecticut: Litchfield County: North of Taconic, Schenob Brook drainage. LLLJ: (AMNH 134483).

**HOUSATONIC RIVER DRAINAGE (EAST BANK)**

39. Massachusetts: Berkshire County: Mill River. LJJ: (AMNH 135602).

40. Connecticut: Litchfield County: Robbins Swamp. LL: (AMNH 122011–13). LJ: (AMNH 122014, AMNH 122017, AMNH 122021). LLJ: (AMNH 122010, AMNH 122018–20, AMNH 122022–32). LLLJ: (AMNH 122016). LLJJ: (AMNH 122015).

41. Connecticut: Litchfield County: Cornwall. LJJ: (AMNH 129421–27). LJJJ: (AMNH 129428–32).

42. Connecticut: Litchfield County: Bantam Lake. LL: (AMNH 122033–34). LLJ: (AMNH 108067, AMNH 122035–41, AMNH 122043–46). LLLJ: (AMNH 122042).

43. Connecticut: Litchfield County: Roxbury. JJ: (AMNH 135604–07). LJJ: (AMNH 135608–18). LJJJ: (AMNH 135619–20).

**CONNECTICUT RIVER DRAINAGE (WEST BANK) AND WEST BANK INDEPENDENT COASTAL DRAINAGES**

44. Connecticut: Litchfield County: North Colebrook. JJ: (AMNH 114053, AMNH 121998–99). LJ: (AMNH 122000). LJJ: (AMNH 122001–08).

45. Connecticut: Hartford County: Bloomfield. LL: (AMNH 114057, AMNH 131653–54). LLJ: (AMNH 108274–75, AMNH 114072–79, AMNH 131655–62).

46. Connecticut: Hartford County: Talcott Mountain. JJ: Avon (AMNH 108266, AMNH 114055, AMNH 124700–01); West Hartford (AMNH 139410). LJJ: Avon (AMNH 108025, AMNH 108268–73, AMNH 114068–69, AMNH 124702, AMNH 131663–64); Farmington (AMNH 114067, AMNH 131665); West Hartford (AMNH 108276, AMNH 134494–97, AMNH 134499–502, AMNH 139411–12). LJJJ: West Hartford (AMNH 134493, AMNH 134498).

47. Connecticut: Hartford County: Southington. JJ: (AMNH 139383–85). LJJ: (AMNH 139386).

48. Connecticut: Hartford County: East Granby. LL: (AMNH 134485). LLJ: (AMNH 134486–87).

49. Connecticut: Hartford County: Suffield. LL: (AMNH 134492). LJ: (AMNH 134491). LLJ: (AMNH 134488–90).
50. Connecticut: New Haven County: Wallingford. LL: (AMNH 114058, AMNH 122047–62, AMNH 122064–70). LLJ: (AMNH 122063, AMNH 122071–75).
51. Connecticut: New Haven County: Meriden. LJ: (AMNH 139387–409).
52. Massachusetts: Franklin County: Gill. JJ: (AMNH 129328–34). LJ: (AMNH 129335–40).
53. Massachusetts: Franklin County: Buckland. JJ: (AMNH 134518).
54. Massachusetts: Hampshire County: Williamsburg. LJ: (AMNH 131638–40).
55. Massachusetts: Hampden County: West Springfield. JJ: (AMNH 129341–43). LJ: (AMNH 129344–46).
56. Massachusetts: Hampden County: Southwick. LJ: (AMNH 129347–53, AMNH 131641–45).

**CONNECTICUT RIVER DRAINAGE (EAST BANK) AND POINTS EAST**

57. Connecticut: Windham and New London Counties: Havey Brook Wetlands. LL: Plainfield (AMNH 134503–10); Griswold (AMNH 134511–13).
58. Connecticut: Tolland County: Scantic River Basin. LL: Somers (AMNH 122076–77, AMNH 129433–35).
59. Massachusetts: Hampden County: Hampden. LJ: (AMNH 114081).
60. Connecticut: Windham County: Plainfield. LL: (AMNH 122078–79, AMNH 129436–45, AMNH 139413–17).
61. Massachusetts: Bristol County: Attleboro. LL: (AMNH 129400–11).
62. Massachusetts: Essex County. LL: Groveland (AMNH 134515, MCZ 107953, MCZ 107959); Hamilton (AMNH 131631); Newbury (MCZ 107956). LJ: Ipswich (MCZ 107960). LLJ: Amesbury (AMNH 134516–17); Georgetown (AMNH 134514, AMNH 131632–34, MCZ 107957–58); Newbury (AMNH 131630); Topsfield (AMNH 122080–85). LLLJ: Topsfield (AMNH 122086).
63. Massachusetts: Franklin County: Quabbin Reservoir. LJ: South of South Athol (AMNH 131635–37).
64. Massachusetts: Hampden County: Wilbraham. LL: (AMNH 129354, AMNH 131646–52).
65. Massachusetts: Middlesex County: Sudbury. LL: (MCZ 107954).
66. Massachusetts: Plymouth County: Hockom-

- ock River drainage. LL: West Bridgewater (AMNH 134519–22).
67. Massachusetts: Worcester County: Harvard. LL: (AMNH 129355–56). LJ: (AMNH 129357–59). LLJ: (AMNH 129360–67).
68. Massachusetts: Worcester County: Northborough. LJ: (AMNH 129368).
69. Massachusetts: Worcester County: East Brookfield. LLJ: (AMNH 129369–71).
70. Massachusetts: Worcester County: East Brookfield River. LL: (AMNH 129372–93). LJ: (AMNH 129394). LLJ: (AMNH 129395–98). LLLJ: (AMNH 129399).

**LONG ISLAND**

71. New York: Suffolk County: Montauk. LL: (AMNH 122159–90, AMNH 129311–13, AMNH 139539–47).

**NEW HAMPSHIRE AND VERMONT  
Connecticut River Drainage**

72. Vermont: Windham County: Vernon. LJ: (AMNH 129454).
73. New Hampshire: Cheshire County: Winchester. JJ: (MCZ 107955).

**MERRIMACK RIVER AND ASSOCIATED COASTAL  
DRAINAGES**

74. New Hampshire: Hillsborough County: Hollis. LL: (UMMZ 176841–42). LJ: (UMMZ 176839–40). LLJ: (UMMZ 176836–37).
75. New Hampshire: Rockingham and Strafford Counties. LL: Rockingham County (MCZ 107621, MCZ 107623, MCZ 107632); Strafford County (MCZ 107619). LJ: Rockingham County (MCZ 107620, MCZ 107624, MCZ 107626–27, MCZ 107629–31, MCZ 107633, MCZ 107952); Strafford County: Durham (AMNH 129446–47).

**LAKE CHAMPLAIN DRAINAGE BASIN**

76. Vermont: Addison County: Snake Mountain, ridge-top. JJ: Bridport (AMNH 129449, AMNH 139436–40, AMNH 140489); Addison (AMNH 140490). LJ: Bridport (AMNH 129450). LLJ: Bridport (AMNH 129451). LJ: Addison (AMNH 140491); Bridport (AMNH 129452–53, AMNH 139441–47, AMNH 139449–51). LJJ: Bridport (AMNH 139448); offspring from triploid AMNH 139446 (AMNH 140445–48).
77. Vermont: Addison County: Snake Mountain, lower slopes and base. LL: (AMNH 139452, AMNH 140509). LJ: (AMNH 139453–55, AMNH 140500–01).
78. Vermont: Addison County: Bridport, ENE junction East Street and Mountain Road Ex-

tension. LLJJ: (AMNH 140492). LLLJ: (AMNH 140493).

79. Vermont: Addison County: Salisbury Swamp. LL: (AMNH 140506–08). LLJ: (AMNH 140494–95). LLLJ: (AMNH 140496–99).

80. Vermont: Addison County: Bridport, Hamilton Hill. LL: (AMNH 139418–20, AMNH 139456–61). LLJ: (AMNH 139421–31). LLLJ: (AMNH 139432–34).

81. Vermont: Addison County: near south end of Buck Mountain. LLJ: (AMNH 140502–04). LLLJ: (AMNH 140505).

82. Vermont: Chittenden County: Shelburne, LaPlatte River drainage. LL: (AMNH 134523–25). LLJ: (AMNH 134526–30). LLLJ: (AMNH 134531–33).

83. Vermont: Addison County: Ferrisburg, Little Otter Creek drainage. LL: (AMNH 134534–38). LLJ: (AMNH 134539–40).

84. Vermont: Grand Isle County: South Hero, South Hero marsh. LL: (AMNH 134543–44). LLJ: (AMNH 134545–50). LLLJ: (AMNH 134551).

85. Vermont: Grand Isle County: Grand Isle, Cedar Swamp. LLJ: (AMNH 134542).

86. Vermont: Addison County: Dead Creek drainage. LLLJ: (AMNH 134541).

87. Vermont: Grand Isle County: North Hero, Pelots Bay marsh. LL: (AMNH 134552–55). LLJ: (AMNH 134556–65). LLLJ: (AMNH 134566).

88. Vermont: Grand Isle County: North Hero, Hazen Point marsh. LL: (AMNH 139475–78). LLJ: (AMNH 134567–73).

89. Vermont: Grand Isle County: North Hero, Hazen Point Road. LL: (AMNH 139479, AMNH 139481). LLJ: (AMNH 139480, AMNH 139482–504); offspring from AMNH 139498 (AMNH 140449–55, AMNH 140458, AMNH 140460, AMNH 140465–66), offspring from AMNH 139504 (AMNH 140468–72); offspring from AMNH 139497 (AMNH 140473–77, AMNH 140479–80, AMNH 140487). LLLJ: Offspring from triploid AMNH 139498 (AMNH 140456–57, AMNH 140459, AMNH 140461–64, AMNH 140467); offspring from triploid AMNH 139497 (AMNH 140478, AMNH 140481–86, AMNH 140488).

90. Vermont: Grand Isle County: North Hero, Birdland. LL: (AMNH 139505–06).

MAINE

91. Aroostook County: US#1 highway between 47°02'44"N, 68°00'09"W and 47°04'19"N, 67°04'20"W. LL: (AMNH 129455). LLJ: (AMNH 129456–60).

92. Aroostook County: Castle Hill Township. LLJ: (MCZ 110571–72).

93. Aroostook County: Chapman Township. LL: (MCZ 110573).

94. Aroostook County: Connor Township, A. LL: (MCZ 110574, MCZ 110576, MCZ 110579, MCZ 110581). LLJ: (MCZ 110575, MCZ 110577–78, MCZ 110580, MCZ 110582).

95. Aroostook County: Connor Township, B. LLJ: MCZ 110583–92).

96. Aroostook County: Cyr Township. LL: (MCZ 110593–94, MCZ 110599, MCZ 110601–02, MCZ 110604–06). LLJ: (MCZ 110595–98, MCZ 110600, MCZ 110603, MCZ 110607–08).

97. Aroostook County: Ludlow Township. LLJ: (MCZ 110615–17).

98. Aroostook County: Masardis Township. LL: (MCZ 110609). LLJ: (MCZ 110611–12). LLLJ: (MCZ 110610).

99. Aroostook County: T10, R4. LL: (MCZ 110613).

100. Hancock County: Ellsworth Township. LLJ: (MCZ 110569–70).

101. Penobscot County: Orono Township. LLJ: (MCZ 110558–59).

102. Penobscot County: North Brewer Township. LL: (MCZ 110564). LLJ: (MCZ 110563, MCZ 110565–66).

103. Penobscot County: South Brewer Township. LLJ: (MCZ 110560).

104. Penobscot County: Hampden Township. LLJ: (MCZ 110567–68).

105. Penobscot County: Hermon Township. LL: (MCZ 110562). LLJ: (MCZ 110561).

106. Waldo County: Monroe Township. LLJ: (MCZ 110614).

**APPENDIX B  
ALLELLE DESIGNATIONS AND  
ERYTHROCYTE SIZES  
FOR NEW YORK AND NEW ENGLAND  
*AMBYSTOMA JEFFERSONIANUM*,  
A. LATERALE AND HYBRIDS**

Unless listed as MCZ or UMMZ, the catalog numbers listed in the "Specimen" columns are AMNH catalog numbers. Sex is listed as female (F), male (M), or juvenile (J). Letter designations for alleles relate to relative mobility (see table 4). No information for an allele is designated by -. A single letter for an allele designation (e.g., A) indicates a homozygous condition for that allele at that locus: a diploid would be AA, a triploid AAA, and a tetraploid AAAA. The specimens are

arranged by site number (see appendix A; figures 1, 2). The allele frequencies are summarized in table 4. Gel systems used to resolve each locus are cited as numbers (Gel number) beneath the locus: (1) Clayton and Tretiak; (2) Tris citrate; (3) Poulik (see table 1). The blood cell measurements ("Blood") are the mean values derived by digitizing and averaging erythrocyte areas for six representative erythrocytes for each individual and are summarized in table 3. This appendix is divided into eight parts (B-1 to B-8) by genotype:

B-1: *Ambystoma jeffersonianum* (JJ)

- B-2: *Ambystoma laterale* (LL)
- B-3: *Ambystoma laterale-jeffersonianum* diploid hybrids (LJ)
- B-4: *Ambystoma* (2) *laterale-jeffersonianum* triploid hybrids (LLJ)
- B-5: *Ambystoma laterale*-(2) *jeffersonianum* triploid hybrids (LJJ)
- B-6: *Ambystoma* (3) *laterale-jeffersonianum* tetraploid hybrids (LLLJ)
- B-7: *Ambystoma* (2) *laterale*-(2) *jeffersonianum* tetraploid hybrids (LLJJ)
- B-8: *Ambystoma laterale*-(3) *jeffersonianum* tetraploid hybrids (LJJJ)

APPENDIX B-1  
*Ambystoma jeffersonianum*

Specimen	Sex	Site	Locus and gel number											
			Adh	AcPh-2	Acon-1	Ldh-1	Mdh-1	Mdh-2	Me-1	Pgi	Pgm-1	Fgm-2	Blood	
			2	2	1	1	1	2	1	3	2	2	(μm <sup>2</sup> )	
1) 122156	F	2	A	B	C	C	BD	B	AB	—	—	B	692	
2) 122157	M	2	A	B	C	C	BD	B	B	—	—	B	663	
3) 122158	F	2	A	B	C	C	B	B	AB	—	—	B	679	
4) 122134	M	5	A	B	C	C	B	B	B	—	BC	B	617	
5) 122135	M	5	A	B	C	C	B	BC	B	—	BC	B	706	
6) 122119	M	6	A	B	C	C	B	BC	A	AB	B	B	612	
7) 131570	M	13	A	B	C	C	B	B	AB	A	BC	B	771	
8) 131571	F	13	A	B	C	C	B	B	B	A	BC	B	746	
9) 131572	M	13	A	B	C	C	B	B	AB	A	B	B	767	
10) 131573	F	13	AC	B	C	C	B	B	B	A	B	B	848	
11) 131574	M	13	A	BC	C	C	B	B	A	AB	BC	B	718	
12) 131575	M	13	A	BC	C	C	B	B	AB	A	BD	B	753	
13) 131576	M	13	A	B	C	C	B	B	AB	A	B	B	766	
14) 131592	F	14	A	B	C	C	B	B	B	A	C	B	707	
15) 131593	M	14	A	BC	BC	C	B	B	B	A	C	B	824	
16) 134461	F	20	A	A	C	C	B	B	B	—	B	B	627	
17) 129290	F	22	A	AB	BC	C	B	B	—	—	BC	B	655	
18) 114051	F	27	A	B	—	C	B	B	—	A	B	B	604	
19) 114052	F	27	A	B	—	C	B	B	—	A	B	B	666	
20) 129316	M	30	A	B	C	C	B	BC	B	—	—	BC	B	667
21) 131615	F	31	A	B	C	C	B	B	AB	A	B	B	708	
22) 131616	M	32	A	AB	C	C	B	B	AB	A	?	B	784	
23) 131617	M	32	A	AB	C	C	B	B	AB	A	B	B	960	
24) 131618	M	32	A	AB	C	C	B	B	AB	AB	B	B	791	
25) 131619	M	32	AC	B	C	C	B	B	AB	B	BC	B	946	
26) 114054	M	34	A	—	C	C	B	B	B	—	—	B	894	
27) 129413	F	35	AC	—	C	C	B	B	AB	—	—	B	780	
28) 129414	M	35	A	—	C	C	B	B	B	—	—	B	678	
29) 129415	M	35	A	—	C	C	B	B	B	—	—	B	621	
30) 129416	F	35	A	—	C	C	B	B	A	—	—	B	688	
31) 129417	M	35	A	—	C	C	B	B	A	—	—	B	717	
32) 135604	M	43	A	B	C	C	B	B	—	AB	B	B	668	
33) 135605	M	43	A	B	C	C	B	B	—	AB	BC	B	685	
34) 135606	F	43	A	B	C	C	B	B	—	AB	BC	B	746	
35) 135607	M	43	A	A	—	C	B	B	AB	A	B	B	754	
36) 114053	?	44	A	—	—	C	B	B	B	—	—	B	807	
37) 121998	M	44	A	—	—	C	B	B	B	—	—	B	800	
38) 121999	F	44	A	B	—	C	B	B	B	—	—	B	737	
39) I08266	F	46	A	B	—	C	B	B	—	—	—	B	677	
40) 114055	M	46	A	—	—	C	B	B	—	—	—	B	603	
41) 124700	M	46	A	—	—	C	B	B	B	—	—	B	—	
42) 124701	F	46	A	—	—	C	B	B	B	BC	—	B	683	
43) 139410	F	46	A	B	C	C	B	B	B	A	B	B	714	
44) 139383	F	47	A	B	C	C	B	B	B	—	A	B	724	
45) 139384	F	47	A	B	C	C	B	B	B	—	A	B	684	
46) 139385	M	47	A	B	C	C	B	B	BC	—	A	B	591	
47) 129328	F	52	A	AB	BC	C	B	B	B	—	—	B	—	
48) 129329	M	52	AC	B	C	C	B	B	BC	—	—	B	—	
49) 129330	F	52	A	B	C	C	B	B	B	AB	—	B	629	
50) 129331	M	52	A	B	C	C	B	B	B	A	—	BC	719	
51) 129332	F	52	A	B	C	C	B	B	B	AB	—	B	733	
52) 129333	F	52	A	B	C	C	B	B	B	AB	—	B	692	
53) 129334	M	52	A	B	C	C	B	B	B	A	—	B	750	
54) 134518	F	53	A	B	C	C	B	B	B	A	—	B	871	
55) 129341	M	55	A	B	C	C	B	B	B	B	—	B	692	
56) 129342	M	55	A	AB	C	C	B	B	B	AB	—	B	730	
57) 129343	M	55	A	B	C	C	B	B	B	B	—	B	701	

**APPENDIX B-1**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 1	Ldh-1 1	Mdh-1 1	Mdh-2 2	Me-1 1	Pgi 3	Pgm-1 2	Pgm-2 2	Blood ( $\mu\text{m}^2$ )	
58) MCZ 107955	M	73	C	B	C	C	—	B	B	—	B	B	589	
59) 129449	F	76	A	AB	B	C	B	B	B	—	C	B	739	
60) 139440	M	76	A	B	B	C	B	B	—	A	B	B	680	
61) 139436	M	76	A	BC	B	C	B	B	B	AB	BC	B	745	
62) 139438	M	76	A	C	B	AC	B	B	B	AB	B	BC	781	
63) 139439	M	76	A	AC	B	C	B	B	AB	AB	BC	B	643	
64) 139437	M	76	A	BC	B	C	B	B	A	A	B	B	852	
65) 140489	M	76	A	C	—	C	B	B	—	—	BC	B	748	
66) 140490	F	76	A	B	—	C	B	B	C	AB	BC	B	957	

There were 12 monomorphic loci: Acon-2(A), Ck-1(A), Ck-2(B), Est-1(B), Got-1(D), Got-2(A), Idh-1(A), Ldh-2(B), Mdh-3(B), Mpi(B), Sod-1(D), 6Pgd(B).

**APPENDIX B-2**  
*Ambystoma laterale*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Got-2 2	Ldh-1 1	Ldh-2 1	Mdh-2 1	Me-1 1	Mpi 1	Pgi 2	Blood ( $\mu\text{m}^2$ )	
1) 129233	F	1	A	A	B	B	B	B	B	A	B	—	604	
2) 129234	F	1	A	A	B	B	C	B	B	A	B	—	680	
3) 129235	F	1	A	A	B	B	BC	AB	B	A	B	—	611	
4) 129238	F	3	A	A	B	B	B	B	B	A	B	C	637	
5) 129239	F	3	A	A	B	B	B	B	B	A	B	—	571	
6) 129240	F	3	AC	AC	B	B	B	B	B	A	B	C	618	
7) 129241	F	3	AC	A	B	B	B	B	B	A	B	—	563	
8) 129278	F	7	C	A	—	B	B	B	B	—	B	A	673	
9) 129279	F	7	C	A	—	B	B	A	B	—	B	A	629	
10) 129280	M	7	AC	A	—	B	B	B	B	—	B	A	660	
11) 131601	M	17	AC	A	B	B	B	A	B	A	B	C	712	
12) 131602	M	17	AC	A	C	B	B	A	B	A	B	B	759	
13) 131603	M	17	AC	A	B	B	B	B	B	A	B	B	603	
14) 131604	F	17	AC	A	B	B	B	AB	B	A	B	B	689	
15) 139538	M	18	A	A	—	B	B	B	B	—	—	C	972	
16) 145056	F	18	—	A	B	B	B	B	B	A	B	C	707	
17) 135598	F	24	A	A	—	B	B	B	B	—	B	C	750	
18) 135599	M	24	A	A	—	B	B	B	B	—	B	C	799	
19) 134465	F	36	AC	A	—	B	B	B	B	A	—	C	752	
20) 139375	M	36	A	A	B	B	B	A	B	—	B	—	677	
21) 122012	F	40	—	—	—	B	B	B	B	—	—	—	643	
22) 122013	F	40	—	—	—	B	B	B	B	—	—	—	630	
23) 122011	J	40	—	—	—	B	B	B	B	—	—	—	588	
24) 122033	F	42	A	A	B	B	B	A	B	A	—	—	693	
25) 122034	F	42	A	—	B	B	B	A	B	B	—	—	671	
26) 114057	F	45	A	—	—	B	B	B	B	—	—	—	675	
27) 131653	M	45	A	A	B	B	B	B	B	A	B	C	816	
28) 131654	M	45	A	A	B	B	B	B	B	A	B	C	609	
29) 134485	F	48	A	A	B	B	B	B	B	A	B	C	742	

**APPENDIX B-2**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Got-2 2	Ldh-1 1	Ldh-2 1	Mdb-2 1	Me-1 1	Mpi 1	Pgi 2	Blood (μm <sup>2</sup> )	
30) 134492	M	49	—	A	B	B	B	B	B	A	—	C	759	
31) 114058	?	50	—	A	BC	B	B	B	B	—	—	C	782	
32) 122047	M	50	AC	A	—	B	B	B	B	B	—	C	523	
33) 122052	F	50	—	A	—	B	B	B	B	—	—	C	658	
34) 122048	F	50	—	A	—	B	B	B	B	B	AB	—	C	660
35) 122049	M	50	—	A	—	B	B	B	B	AB	—	C	731	
36) 122050	F	50	—	A	—	B	B	B	B	B	B	C	704	
37) 122051	M	50	—	A	—	B	B	B	B	B	AB	C	715	
38) 122054	F	50	C	A	BC	B	B	A	B	A	—	C	718	
39) 122056	J	50	—	A	B	B	B	B	B	A	A	C	492	
40) 122058	J	50	AC	A	C	B	B	B	B	A	—	C	616	
41) 122057	J	50	—	A	—	B	B	A	B	—	A	C	618	
42) 122055	J	50	AC	A	C	B	B	B	B	A	A	C	652	
43) 122061	F	50	C	A	B	B	B	B	B	A	A	C	713	
44) 122062	F	50	—	A	—	B	B	B	B	—	B	C	658	
45) 122067	F	50	AC	A	B	B	B	B	B	A	A	C	612	
46) 122064	J	50	—	A	—	B	B	B	B	—	B	C	632	
47) 122065	J	50	—	A	—	B	B	AB	B	—	AB	C	677	
48) 122070	F	50	AC	A	B	B	B	B	B	B	—	C	677	
49) 122059	F	50	A	A	B	B	B	B	BC	A	A	C	683	
50) 122060	F	50	—	A	—	B	B	B	B	—	AB	C	629	
51) 122066	F	50	—	A	—	B	B	B	B	—	AB	C	655	
52) 122068	F	50	—	A	—	B	B	AB	B	—	B	C	625	
53) 122069	F	50	—	A	—	B	B	B	B	—	B	C	630	
54) 122053	F	50	AC	A	C	B	B	B	B	AB	—	C	625	
55) 134503	M	57	—	A	B	B	B	A	B	A	—	C	761	
56) 134504	M	57	—	A	B	B	B	B	B	A	—	C	743	
57) 134511	F	57	—	A	B	B	B	B	B	A	—	C	688	
58) 134512	F	57	—	A	B	B	B	B	B	A	—	C	842	
59) 134513	F	57	—	A	B	B	B	B	B	A	—	C	675	
60) 134505	F	57	—	A	B	B	B	B	B	A	—	C	854	
61) 134506	F	57	—	A	B	B	B	B	B	A	—	C	787	
62) 134507	M	57	—	A	B	B	B	B	B	A	—	C	682	
63) 134508	F	57	—	A	B	B	B	B	B	A	—	C	765	
64) 134509	F	57	—	A	B	B	B	A	B	A	—	C	734	
65) 134510	F	57	—	A	B	B	B	AB	B	A	—	C	772	
66) 122078	J	60	A	AC	B	B	B	A	B	A	B	C	599	
67) 122079	J	60	—	A	B	B	B	A	B	A	B	—	514	
68) 129437	F	60	C	A	B	B	B	A	B	A	B	—	603	
69) 129438	F	60	AC	AC	B	B	B	A	B	A	B	—	618	
70) 129436	F	60	C	C	B	B	B	A	B	A	B	—	588	
71) 129441	M	60	C	—	B	B	B	A	B	A	B	A	608	
72) 129439	F	60	C	A	B	B	B	A	B	A	B	A	660	
73) 129440	M	60	AC	A	B	B	B	A	B	A	B	AC	583	
74) 129442	M	60	—	A	B	B	B	A	B	A	B	A	623	
75) 129443	M	60	AC	A	B	B	B	A	B	A	B	A	640	
76) 129444	M	60	—	A	B	B	B	A	B	A	B	A	642	
77) 129445	M	60	C	—	B	B	B	A	B	A	B	A	653	
78) 139413	F	60	C	A	B	B	B	A	B	A	B	A	629	
79) 139414	F	60	C	A	B	B	B	A	B	A	B	A	730	
80) 139415	F	60	C	A	B	B	B	A	B	A	B	A	653	
81) 139416	M	60	C	A	B	B	B	A	B	A	B	A	732	
82) 139417	F	60	C	A	B	B	B	A	B	A	B	A	724	
83) 129400	F	61	C	A	B	B	B	A	B	A	—	660		
84) 129401	F	61	C	A	B	B	B	A	BC	A	—	658		
85) 129402	M	61	C	A	B	B	B	A	B	A	—	671		
86) 129403	F	61	AC	A	B	B	B	A	B	A	—	612		

**APPENDIX B-2**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number												Blood ( $\mu\text{m}^2$ )
			Adh 2	AcPh-2 2	Acon-1 2	Got-2 2	Ldh-1 1	Ldh-2 1	Mdh-2 1	Me-1 1	Mpi 1	Pgi 2			
87)	129404	M	61	C	A	B	B	A	B	A	—	—	—	—	626
88)	129405	M	61	AC	A	B	B	A	BC	A	—	—	—	—	596
89)	129406	M	61	C	A	B	B	A	B	A	—	—	—	—	661
90)	129407	M	61	C	A	B	B	A	BC	A	—	—	—	—	542
91)	129408	F	61	C	A	B	B	A	BC	A	—	—	—	—	641
92)	129409	F	61	C	A	B	B	A	BC	A	—	—	—	—	407
93)	129410	M	61	C	A	B	B	B	AB	B	A	—	—	—	601
94)	129411	F	61	C	A	B	B	B	A	B	A	—	—	—	676
95)	MCZ 107953	F	62	—	—	—	B	B	A	B	—	—	—	—	570
96)	MCZ 107956	F	62	—	—	B	B	B	A	B	—	—	—	C	650
97)	MCZ 107959	?	62	—	—	B	—	—	—	—	A	—	—	—	—
98)	131631	F	62	AC	A	B	B	B	AB	B	A	B	C	C	690
99)	134515	M	62	A	A	B	B	B	B	B	A	—	C	C	774
100)	MCZ 107954	J	65	—	—	B	B	B	A	B	B	A	B	A	566
101)	134519	M	66	—	A	B	B	B	B	B	B	A	C	C	812
102)	134520	M	66	—	A	B	B	B	B	B	B	A	—	C	776
103)	134521	M	66	—	A	B	B	B	B	B	B	A	—	C	768
104)	134522	F	66	—	A	B	B	B	B	B	B	A	—	C	764
105)	129355	M	67	A	AC	—	B	B	A	B	A	—	—	—	997
106)	129356	F	67	A	AC	—	B	B	A	B	A	—	—	—	921
107)	129372	F	70	C	—	B	B	B	A	B	A	B	—	—	597
108)	129374	M	70	C	—	B	B	B	A	B	A	B	C	C	635
109)	129373	M	70	C	—	B	B	B	A	B	A	B	—	C	647
110)	129375	M	70	C	—	B	B	B	A	B	A	B	—	C	590
111)	129376	F	70	C	—	B	B	B	A	B	A	B	—	C	582
112)	129377	J	70	AC	—	B	B	B	A	B	A	B	—	C	617
113)	129378	M	70	C	—	B	B	B	A	B	A	B	—	C	664
114)	129379	F	70	AC	—	B	B	B	A	B	A	B	—	C	575
115)	129380	F	70	AC	—	B	B	B	A	B	A	B	—	C	631
116)	129381	M	70	C	—	B	B	B	A	B	A	B	—	C	668
117)	129382	F	70	C	—	B	B	B	A	B	A	B	—	C	646
118)	129383	F	70	C	—	B	B	B	A	B	A	B	—	C	700
119)	129384	F	70	C	—	B	B	B	A	B	A	B	—	C	620
120)	129385	M	70	C	—	B	B	B	A	B	A	B	—	C	695
121)	129386	M	70	AC	—	B	B	B	A	B	A	B	—	C	632
122)	129387	F	70	C	—	B	B	B	A	B	A	B	—	—	615
123)	129388	M	70	C	—	B	B	B	A	B	A	B	—	—	535
124)	129389	F	70	C	—	—	B	B	A	B	A	B	—	—	640
125)	129390	M	70	AC	—	B	B	B	A	B	A	B	—	AC	614
126)	129391	F	70	C	—	B	B	B	A	B	A	B	—	—	666
127)	129392	M	70	AC	—	B	B	B	A	B	A	B	—	C	656
128)	129393	M	70	C	—	B	B	B	A	B	A	B	—	C	593
129)	122159	?	71	AC	—	—	B	B	A	B	—	—	—	—	—
130)	122160	M	71	—	—	—	B	B	A	B	—	—	—	—	—
131)	122178	M	71	—	—	—	B	B	A	B	—	—	—	—	—
132)	122179	F	71	—	—	—	B	B	A	B	AB	—	—	—	—
133)	122180	F	71	—	—	—	B	B	A	B	AB	—	—	—	—
134)	122174	F	71	—	—	—	B	B	A	B	A	—	—	—	—
135)	122175	M	71	—	—	—	B	B	A	B	A	—	—	—	—
136)	122176	F	71	—	—	—	B	B	A	B	A	—	—	—	—
137)	122177	M	71	—	—	—	B	B	A	B	A	—	—	—	—
138)	122165	F	71	—	—	—	B	B	A	B	A	—	—	—	—
139)	122166	M	71	—	—	—	B	B	A	B	A	—	—	—	—
140)	122167	F	71	—	—	—	B	B	A	B	AB	—	—	—	680
141)	122161	F	71	—	—	—	B	B	A	B	—	—	—	—	—
142)	122163	F	71	—	—	—	B	B	A	B	—	—	—	—	672
143)	122164	M	71	—	A	—	B	B	A	B	—	—	—	—	702

APPENDIX B-2  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Got-2 2	Ldh-1 1	Ldh-2 1	Mdh-2 1	Mc-1 1	Mpi 1	Pgi 2	Blood ( $\mu\text{m}^2$ )	
144) 122168	M	71	—	—	—	B	B	A	B	B	B	—	—	
145) 122169	M	71	—	—	—	B	B	A	B	B	AB	—	—	
146) 122170	M	71	—	—	—	B	B	A	B	AB	B	—	—	
147) 122171	F	71	—	—	—	B	B	A	B	B	AB	—	—	
148) 122172	M	71	—	—	—	B	B	A	B	B	B	—	—	
149) 122173	M	71	—	—	—	B	B	A	B	B	B	—	—	
150) 122186	M	71	A	A	B	B	B	A	B	A	—	—	611	
151) 122187	M	71	C	A	B	B	B	A	B	A	—	—	717	
152) 122188	M	71	AC	A	B	B	B	A	B	B	—	—	704	
153) 122189	M	71	AC	A	BC	B	B	A	B	AB	—	—	656	
154) 122190	M	71	A	C	B	B	B	A	B	A	—	—	670	
155) 122181	M	71	C	A	B	B	B	A	B	B	—	—	626	
156) 122182	M	71	—	C	B	B	B	A	B	A	—	—	611	
157) 122183	M	71	C	A	B	B	B	A	B	A	—	—	663	
158) 122184	M	71	A	AC	B	B	B	A	B	A	—	—	697	
159) 122185	M	71	AC	AC	BC	B	B	A	B	AB	—	—	565	
160) 129312	M	71	C	—	B	B	B	A	B	A	—	—	743	
161) 129311	M	71	C	—	B	B	B	A	B	A	—	—	616	
162) 129313	M	71	C	—	B	B	B	A	B	A	—	—	677	
163) 139539	F	71	—	—	—	B	B	A	B	—	—	—	644	
164) 139540	F	71	—	—	—	B	B	A	B	—	—	—	701	
165) 139541	F	71	—	—	—	B	B	A	B	—	—	—	673	
166) 139542	F	71	—	—	—	B	B	A	B	—	—	—	—	
167) 139543	F	71	—	—	—	B	B	A	B	—	—	—	—	
168) 139544	F	71	—	—	—	B	B	A	B	—	—	—	717	
169) 139545	F	71	—	—	—	B	B	A	B	—	—	—	698	
170) 139546	F	71	—	—	—	B	B	A	B	—	—	—	819	
171) 139547	F	71	—	—	—	B	B	A	B	—	—	—	—	
172) UMMZ 176841	?	74	—	—	—	B	B	AB	B	—	A	—	—	
173) UMMZ 176842	F	74	—	—	—	B	B	A	B	—	—	—	721	
174) MCZ 107619	F	75	—	—	—	B	B	A	B	A	—	—	708	
175) MCZ 107621	F	75	—	—	—	B	B	A	B	A	—	—	630	
176) MCZ 107623	F	75	—	—	—	B	B	A	B	A	—	—	—	
177) MCZ 107632	M	75	—	—	—	B	B	A	B	A	—	—	638	
178) 139452	M	77	—	A	—	B	B	B	B	A	—	C	686	
179) 140509	M	77	A	A	—	B	B	B	B	B	—	C	862	
180) 140506	M	79	A	C	—	B	B	A	B	—	B	—	827	
181) 140507	M	79	A	A	—	BC	B	A	B	—	B	—	752	
182) 140508	M	79	C	A	—	B	B	AB	B	—	B	C	626	
183) 139456	F	80	—	A	—	B	B	AB	B	—	A	C	524	
184) 139457	F	80	—	A	—	B	B	AB	B	—	—	C	678	
185) 139458	F	80	—	A	—	B	B	A	B	—	B	C	724	
186) 139459	F	80	—	A	—	B	B	AB	B	—	B	C	543	
187) 139460	F	80	—	A	—	B	B	AB	B	A	—	C	586	
188) 139461	F	80	—	C	—	B	B	A	B	A	—	C	651	
189) 139418	F	80	—	—	—	B	B	A	B	A	—	C	632	
190) 139419	M	80	—	A	—	B	B	A	B	A	—	C	968	
191) 139420	M	80	—	A	—	B	B	A	B	—	B	C	734	
192) 134523	M	82	C	A	B	B	B	B	B	B	—	C	642	
193) 134524	M	82	AC	A	B	B	B	B	B	B	—	C	698	
194) 134525	M	82	C	A	B	B	B	B	B	B	—	C	621	
195) 134534	F	83	A	A	B	B	B	A	B	B	—	B	898	
196) 134535	F	83	A	A	B	B	B	A	B	A	B	C	777	
197) 134536	M	83	—	A	—	B	B	A	B	A	—	C	738	
198) 134537	M	83	—	A	—	B	B	A	B	A	—	C	720	
199) 134538	M	83	—	A	—	B	B	A	B	A	—	C	769	
200) 134543	M	84	—	A	—	B	B	B	B	A	—	C	791	

**APPENDIX B-2**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Got-2 2	Ldh-1 1	Ldh-2 1	Mdh-2 1	Me-1 1	Mpi 1	Pgi 2	Blood ( $\mu\text{m}^2$ )	
201) 134544	F	84	—	A	B	B	B	B	B	A	—	C	832	
202) 134552	F	87	A	A	B	B	B	A	B	A	—	C	776	
203) 134553	F	87	A	A	B	B	B	A	B	—	—	BC	946	
204) 134554	M	87	A	A	B	B	B	A	B	A	—	C	763	
205) 134555	F	87	A	A	B	B	B	A	B	A	—	C	760	
206) 139475	F	88	AC	A	B	B	B	A	B	A	B	C	637	
207) 139476	M	88	A	A	B	B	B	A	B	A	B	C	—	
208) 139477	M	88	—	A	B	B	B	A	B	A	—	C	665	
209) 139478	M	88	—	A	B	B	B	A	B	A	—	C	591	
210) 139479	M	89	—	A	—	B	B	A	B	—	B	C	700	
211) 139481	M	89	—	A	—	B	B	A	B	A	—	—	684	
212) 139505	M	90	AC	A	—	B	B	A	B	—	B	C	762	
213) 139506	F	90	—	A	—	B	B	A	B	A	—	C	697	
214) 129455	F	91	C	—	B	B	B	A	B	A	B	C	657	
215) MCZ 110573	M	93	C	A	—	B	B	AB	B	A	B	B	751	
216) MCZ 110574	M	94	—	A	—	B	B	A	B	B	B	C	735	
217) MCZ 110576	F	94	—	A	B	B	B	B	B	—	B	C	831	
218) MCZ 110579	M	94	—	A	B	B	B	B	B	—	B	C	785	
219) MCZ 110581	F	94	—	A	B	B	B	AB	B	—	B	C	847	
220) MCZ 110599	F	96	A	—	B	B	B	AB	B	A	AB	C	—	
221) MCZ 110593	F	96	A	A	B	B	B	AB	B	A	B	C	842	
222) MCZ 110594	F	96	A	A	B	B	B	AB	B	A	B	C	690	
223) MCZ 110601	F	96	AC	A	B	B	B	AB	B	A	B	C	755	
224) MCZ 110602	M	96	C	A	B	B	B	B	B	A	B	C	674	
225) MCZ 110604	F	96	A	A	B	B	B	A	B	AB	B	C	726	
226) MCZ 110605	F	96	C	A	B	B	B	AB	B	A	B	C	731	
227) MCZ 110606	F	96	A	A	B	B	B	A	B	AB	B	C	689	
228) MCZ 110609	M	98	C	A	B	B	B	A	B	A	—	A	778	
229) MCZ 110613	F	99	C	A	B	B	B	AB	B	A	B	A	768	
230) MCZ 110564	F	102	C	A	B	B	B	AB	B	A	B	B	744	
231) MCZ 110562	F	105	C	A	—	B	B	AB	B	A	B	C	754	

There were 12 monomorphic loci: Acon-2(B), Ck-1(A), Ck-2(A), Est-1(A), Got-1(B), Idh-1(B), Mdh-1(D), Mdh-3(B), Pgm-1(B), Pgm-2(B), Sod-1(B), 6Pgd(B).

**APPENDIX B-3**  
*Ambystoma laterale*  $\times$  *A. jeffersonianum* Diploid Hybrids

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Ck-1 1	Ck-2 1	Est-1 1	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
1) 129236	F	1	A	AB	BC	A	AB	—	BD	AB	AB	BC	A	
2) 129242	F	3	A	AC	B	A	AB	—	BD	B	AB	B	A	
3) 129243	F	3	A	A	B	A	AB	—	BD	AB	AB	B	A	
4) 129244	F	3	A	AC	B	A	AB	—	BD	AB	AB	BC	AB	
5) 129245	F	3	A	AC	B	A	AB	—	BD	AB	AB	BC	AB	
6) 129246	F	3	A	AC	B	A	AB	—	BD	AB	AB	BC	AB	
7) 129247	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB	

APPENDIX B-3  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh	AcPh-2	Acon-1	Ck-1	Ck-2	Est-1	Got-1	Got-2	Idh-1	Ldh-1	Ldh-2	
			2	2	2	1	1	1	3	2	1	1	1	1
8)	129248	F	3	A	AC	B	A	AB	—	BD	AB	AB	BC	AB
9)	129249	F	3	A	A	B	A	AB	—	BD	AB	AB	BC	AB
10)	129250	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
11)	129251	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
12)	129252	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
13)	129253	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
14)	129254	F	3	A	A	B	A	AB	—	BD	AB	AB	BC	B
15)	129255	F	3	A	A	B	A	AB	—	BD	AB	AB	BC	A
16)	129256	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
17)	129257	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
18)	129258	F	3	AC	AC	B	A	AB	—	BD	AB	B	BC	AB
19)	129259	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
20)	129260	F	3	AC	A	B	A	AB	—	BD	AB	AB	BC	AB
21)	129261	F	3	AC	AC	B	A	AB	—	BD	AB	AB	BC	AB
22)	129262	F	3	AC	AC	BC	A	AB	—	BD	AB	AB	BC	AB
23)	129281	F	7	C	A	—	A	AB	—	BD	AB	AB	BC	AB
24)	129282	F	7	C	A	—	A	AB	—	BD	AB	AB	BC	A
25)	129283	F	7	C	A	—	A	AB	—	BD	AB	AB	BC	B
26)	129284	F	7	C	AB	—	A	AB	—	BD	AB	AB	BC	AB
27)	122114	F	12	C	AB	B	—	—	—	BD	AB	AB	BC	B
28)	122104	F	12	A	AB	B	—	—	—	BD	AB	AB	BC	B
29)	131577	F	13	AC	AB	BC	A	AB	A	BD	AB	AB	BC	AB
30)	131578	F	13	A	AB	BC	A	AB	AB	BD	AB	AB	BC	AB
31)	131579	F	13	AC	AB	BC	A	AB	AB	BD	AB	AB	BC	A
32)	131580	F	13	A	A	C	A	AB	—	BD	AB	AB	BC	AB
33)	131581	F	13	AC	AB	BC	A	AB	BD	AB	AB	BC	AB	AB
34)	131582	F	13	AC	AB	BC	A	AB	BD	AB	AB	BC	AB	AB
35)	131583	F	13	A	A	C	A	AB	—	BD	AB	AB	BC	AB
36)	131591	F	13	AC	AB	BC	A	AB	AB	BD	AB	AB	BC	AB
37)	131605	F	17	AC	AB	BC	A	AB	AB	BD	AB	AB	BC	AB
38)	131606	F	17	C	AB	BC	A	AB	AB	BD	AB	AB	BC	AB
39)	131607	F	17	A	AB	BC	A	AB	AB	BD	AB	AB	BC	AB
40)	145036	F	18	—	AB	BC	A	A	—	BD	AB	AB	BC	B
41)	145042	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	B
42)	145048	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	AB
43)	145049	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	AB
44)	145051	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	B
45)	145052	F	18	—	A	BC	A	AB	—	BD	AB	AB	BC	AB
46)	145054	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	B
47)	145057	F	18	—	A	BC	A	AB	—	BD	AB	AB	BC	B
48)	145060	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	AB
49)	145061	F	18	—	AB	BC	A	AB	—	BD	AB	AB	BC	AB
50)	129301	F	20	—	AB	BC	AB	AB	—	BD	AB	AB	BC	B
51)	129302	F	20	—	AB	BC	AB	AB	—	BD	AB	AB	BC	B
52)	129303	F	20	—	AB	BC	AB	AB	—	BD	AB	AB	BC	AB
53)	129294	F	22	A	A	C	A	AB	—	BD	AB	AB	BC	AB
54)	129293	F	22	A	A	C	A	AB	—	BD	AB	AB	BC	AB
55)	129295	F	22	A	A	C	A	AB	—	BD	AB	AB	BC	AB
56)	129291	F	22	A	A	BC	A	AB	—	BD	AB	AB	BC	AB
57)	121995	F	23	—	—	—	A	AB	—	BD	AB	AB	B	AB
58)	121996	F	23	—	—	—	A	AB	—	BD	AB	AB	BC	B
59)	121992	F	23	—	—	—	A	AB	—	BD	AB	A	BC	B
60)	121997	F	23	—	—	—	A	AB	—	BD	AB	A	BC	B
61)	121990	F	23	—	—	—	A	AB	—	BD	AB	AB	BC	B
62)	121994	F	23	—	—	—	A	AB	—	BD	AB	—	BC	B
63)	121993	F	23	—	—	—	A	AB	—	BD	AB	AB	BC	B
64)	121985	F	23	A	AC	?	A	AB	—	BD	AB	AB	BC	B

APPENDIX B-3  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh-2	AcPh-2	Acon-1-2	Ck-1-1	Ck-2-1	Est-1-1	Got-1-3	Got-2-2	Idh-1-1	Ldh-1-1	Ldh-2-1	
65) 121986	F	23	A	AC	BC	A	AB	—	BD	AB	AB	BC	B	
66) 121973	F	26	A	AB	B	—	—	—	BD	AB	AB	BC	A	
67) 129319	F	30	A	AB	C	AB	AB	AB	BD	AB	AB	BC	AB	
68) 129320	F	30	A	B	BC	AB	AB	AB	BD	AB	AB	BC	AB	
69) 129321	F	30	A	AB	BC	AB	AB	AB	BD	AB	AB	BC	AB	
70) 129317	F	30	AC	A	C	A	AB	AB	BD	AB	AB	BC	A	
71) 129318	F	30	A	AB	BC	AB	AB	AB	BD	AB	AB	BC	A	
72) 129418	F	35	A	—	C	—	AB	—	BD	AB	AB	BC	B	
73) 122021	F	40	—	—	—	—	—	AB	BD	—	—	B	—	
74) 122014	F	40	—	—	—	—	—	AB	BD	—	—	B	—	
75) 122017	F	40	—	—	—	—	—	AB	BD	—	—	BC	—	
76) 122000	F	44	—	—	—	—	—	AB	BD	—	—	BC	B	
77) 134491	F	49	—	AB	BC	—	AB	—	BD	AB	AB	BC	B	
78) MCZ 107960	M	62	—	—	BC	—	—	—	BD	AB	B	BC	—	
79) 129357	F	67	AC	AB	—	—	AB	AB	BD	AB	AB	BC	AB	
80) 129358	F	67	AC	AB	—	—	AB	AB	BD	AB	AB	BC	A	
81) 129359	F	67	AC	A	—	—	AB	AB	BD	AB	AB	BC	AB	
82) 129368	F	68	AC	AB	—	—	AB	AB	BD	AB	AB	BC	BC	
83) 129394	M	70	C	—	B	—	AB	—	BD	AB	AB	BC	AB	
84) 129450	F	76	AC	AB	B	—	AB	AB	BD	AB	AB	BC	AB	

Specimen	Sex	Site	Locus and gel number								Blood ( $\mu\text{m}^2$ )
			Mdh-1-1	Mdh-2-1	Me-1-1	Mpi-1	Pgi-2	Pgm-1-1	Sod-1-2		
1) 129236	F	1	BD	B	AB	BC	—	BC	BD	628	
2) 129242	F	3	BD	BC	AB	BC	—	—	BD	702	
3) 129243	F	3	BD	B	AB	BC	—	—	BD	740	
4) 129244	F	3	BD	B	AB	BC	—	—	BD	710	
5) 129245	F	3	BD	B	A	BC	—	—	BD	684	
6) 129246	F	3	BD	B	AB	BC	—	—	BD	700	
7) 129247	F	3	BD	B	AB	BC	AC	BC	BD	666	
8) 129248	F	3	BD	B	AB	BC	AC	B	BD	618	
9) 129249	F	3	BD	B	AB	BC	—	BC	BD	669	
10) 129250	F	3	BD	B	AB	BC	—	BC	BD	696	
11) 129251	F	3	BD	B	AB	BC	AC	BC	BD	609	
12) 129252	F	3	BD	B	AB	BC	AC	BC	BD	653	
13) 129253	F	3	BD	B	AB	BC	—	BC	BD	652	
14) 129254	F	3	BD	B	AB	BC	AC	B	BD	787	
15) 129255	F	3	BD	B	AB	BC	—	BC	BD	711	
16) 129256	F	3	BD	B	AB	BC	AC	BC	BD	644	
17) 129257	F	3	BD	B	AB	BC	—	BC	BD	641	
18) 129258	F	3	BD	B	AB	BC	—	BC	BD	741	
19) 129259	F	3	BD	B	AB	BC	AC	BC	BD	584	
20) 129260	F	3	BD	B	AB	BC	AC	BC	BD	714	
21) 129261	F	3	BD	B	AB	BC	AC	BC	BD	571	
22) 129262	F	3	BD	B	AB	BC	AC	BC	BD	618	
23) 129281	F	7	BD	B	—	BC	A	B	BD	700	
24) 129282	F	7	BD	B	—	BC	A	B	BD	720	
25) 129283	F	7	BD	B	—	BC	A	B	BD	653	
26) 129284	F	7	BD	B	—	BC	A	B	BD	675	
27) 122114	F	12	BD	BC	B	—	—	C	BD	687	
28) 122104	F	12	BD	B	B	—	—	B	BD	689	
29) 131577	F	13	BD	B	AB	BC	AB	B	BD	744	
30) 131578	F	13	BD	B	AB	AB	AB	B	BD	—	
31) 131579	F	13	BD	B	AB	AB	AC	B	BD	716	
32) 131580	F	13	BD	B	AB	AB	AB	BC	BD	780	
33) 131581	F	13	BD	B	AB	AB	AC	B	BD	—	

APPENDIX B-3  
(Continued)

Specimen	Sex	Site	Locus and gel number								Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Me-1 1	Mpi 1	Pgi 2	Pgm-1 1	Sod-1 2		
34)	131582	F	13	BD	B	AB	BC	AB	B	BD	758
35)	131583	F	13	BD	B	AB	AB	AB	B	BD	763
36)	131591	F	13	BD	B	AB	BC	AB	B	BD	722
37)	131605	F	17	BD	B	AB	AB	AB	B	BD	784
38)	131606	F	17	BD	B	AB	AB	AB	B	BD	761
39)	131607	F	17	BD	B	AB	AB	AB	B	BD	744
40)	145036	F	18	BD	B	AB	AB	AC	B	BD	819
41)	145042	F	18	BD	B	AB	AB	AC	B	BD	807
42)	145048	F	18	BD	B	AB	AB	AC	B	BD	765
43)	145049	F	18	BD	B	AB	AB	AC	B	BD	814
44)	145051	F	18	BD	B	AB	AB	AC	B	BD	861
45)	145052	F	18	BD	B	AB	AB	AC	B	BD	883
46)	145054	F	18	BD	B	AB	AB	AC	B	BD	614
47)	145057	F	18	BD	B	AB	AB	AC	B	BD	788
48)	145060	F	18	BD	B	AB	AB	AC	B	BD	723
49)	145061	F	18	BD	B	AB	AB	AC	B	BD	758
50)	129301	F	20	BD	B	AB	—	—	B	BD	809
51)	129302	F	20	BD	B	AB	—	—	B	BD	685
52)	129303	F	20	BD	B	AB	—	—	B	BD	739
53)	129294	F	22	BD	B	—	BC	—	BC	BD	684
54)	129293	F	22	BD	B	—	BC	—	BC	BD	637
55)	129295	F	22	BD	B	—	BC	—	B	BD	760
56)	129291	F	22	BD	B	—	BC	—	B	BD	647
57)	121995	F	23	BD	B	B	—	—	B	BD	743
58)	121996	F	23	BD	B	B	—	—	BC	BD	693
59)	121992	F	23	BD	B	AB	—	—	BC	BD	714
60)	121997	F	23	BD	B	AB	—	—	BC	BD	678
61)	121990	F	23	BD	B	B	—	—	B	BD	630
62)	121994	F	23	BD	B	AB	—	—	—	BD	731
63)	121993	F	23	BD	B	—	—	—	BC	BD	699
64)	121985	F	23	BD	B	A	—	—	BC	BD	659
65)	121986	F	23	BD	B	AB	—	—	BC	BD	767
66)	121973	F	26	BD	B	A	—	—	—	BD	666
67)	129319	F	30	BD	B	AB	—	—	B	BD	—
68)	129320	F	30	BD	B	AB	—	—	B	BD	655
69)	129321	F	30	BD	B	AB	—	—	B	BD	670
70)	129317	F	30	BD	BC	AB	—	—	B	BD	763
71)	129318	F	30	BD	B	AB	—	—	B	BD	682
72)	129418	F	35	BD	B	AB	BC	—	—	BD	656
73)	122021	F	40	BD	B	—	—	—	—	BD	704
74)	122014	F	40	BD	B	—	—	—	—	BD	696
75)	122017	F	40	BD	B	—	—	—	—	BD	636
76)	122000	F	44	BD	B	B	—	—	—	BD	686
77)	134491	F	49	BD	B	AB	—	AC	BC	BD	730
78)	MCZ 107960	M	62	BD	B	AB	—	—	B	BD	599
79)	129357	F	67	BD	B	AB	—	—	B	BD	592
80)	129358	F	67	BD	B	AB	—	—	B	BD	691
81)	129359	F	67	BD	B	AB	—	—	B	BD	—
82)	129368	F	68	BD	B	AB	—	—	B	BD	609
83)	129394	M	70	BD	B	AB	AB	—	B	BD	764
84)	129450	F	76	BD	B	AB	—	—	BC	BD	663

There were four monomorphic loci: Acon-2(A), Mdh-3(B), Pgm-2(B), 6Pgd(B).

APPENDIX B-4  
*Ambystoma (2) laterale-jeffersonianum* Triploid Hybrids

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Ck-1 1	Ck-2 1	Est-1 1	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
1) 129237	F	1	A	AAB	BBC	A	AAB	—	BBD	ABB	ABB	C	B	
2) 129267	F	3	A	A	B	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
3) 129263	F	3	A	A	B	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
4) 129268	F	3	A	A	B	A	A	—	BBD	ABB	B	B	A	
5) 129266	F	3	ACC	ACC	B	A	AAB	—	BBD	ABB	ABB	BBC	B	
6) 129269	F	3	A	A	B	A	AAB	—	BBD	ABB	ABB	BBC	B	
7) 129270	F	3	ACC	AAC	B	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
8) 129271	F	3	AAC	AAC	B	A	AAB	—	BBD	ABB	ABB	BBC	B	
9) 129272	F	3	AAC	A	B	A	AAB	—	BBD	ABB	ABB	BBC	A	
10) 129273	F	3	A	A	B	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
11) 129274	F	3	AAC	A	B	A	AAB	—	BBD	ABB	B	B	A	
12) 129275	F	3	AAC	AAC	B	A	AAB	—	BBD	ABB	ABB	BBC	B	
13) 129264	F	3	AAC	AAC	B	A	A	—	BBD	ABB	ABB	BBC	A	
14) 129265	F	3	AAC	A	B	A	AAB	—	BBD	ABB	ABB	BBC	B	
15) 129285	F	7	AAC	A	—	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
16) 129286	F	7	AAC	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
17) 131600	F	15	AAC	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
18) 122117	F	16	ACC	A	—	A	AAB	—	BBD	ABB	ABB	BBC	A	
19) 122118	F	16	ACC	A	—	A	AAB	—	BBD	ABB	ABB	BBC	A	
20) 131608	F	17	ACC	A	C	A	AAB	—	BBD	ABB	ABB	BCC	ABB	
21) 131609	F	17	ACC	A	BBC	A	AAB	A	BBD	ABB	ABB	BBC	AAB	
22) 131610	F	17	ACC	AAB	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	B	
23) 131611	F	17	ACC	AAB	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	
24) 131612	F	17	AAC	A	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	ABB	
25) 131613	M	17	A	AAB	BBC	A	AAB	AAB	BBD	ABB	B	BBC	A	
26) 135600	F	18	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	B	
27) 140443	F	18	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
28) 140444	F	18	—	A	—	A	AAB	—	BBD	ABB	ABB	BCC	ABB	
29) 145035	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
30) 145037	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
31) 145038	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
32) 145039	F	18	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
33) 145040	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
34) 145041	F	18	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
35) 145043	F	18	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
36) 145044	F	18	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
37) 145045	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	AAB	BBC	B	
38) 145046	F	18	—	A	BBC	A	ABB	—	BBD	ABB	ABB	BBC	AAB	
39) 145050	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	B	BBC	B	
40) 145053	F	18	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
41) 145055	F	18	—	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
42) 145059	F	18	—	A	BBC	A	ABB	—	BBD	ABB	ABB	BBC	B	
43) 121977	F	23	A	—	—	A	AAB	—	BBD	ABB	ABB	B	B	
44) 121979	F	23	A	—	—	A	AAB	—	ABD	ABB	ABB	B	B	
45) 121980	F	23	A	—	—	A	AAB	—	BBD	ABB	AAB	BBC	B	
46) 121978	F	23	A	—	—	A	AAB	—	ABD	ABB	ABB	B	B	
47) 121974	F	23	A	—	—	A	AAB	—	BBD	ABB	ABB	B	B	
48) 121975	F	23	A	—	—	A	AAB	—	BBD	ABB	ABB	B	ABB	
49) 121976	F	23	A	—	—	A	AAB	—	BBD	ABB	ABB	B	B	
50) 121981	F	23	A	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
51) 121982	F	23	A	AAB	—	A	AAB	—	ABD	ABB	AAB	BBC	B	
52) 121983	F	23	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	B	
53) 121984	F	23	AAC	AAB	BCC	A	AAB	—	ABD	ABB	ABB	BBC	B	
54) 121987	F	23	A	AAB	BCC	A	AAB	—	ABD	ABB	ABB	BBC	B	
55) 121989	F	23	AAC	AAB	?	A	AAB	—	ABD	ABB	ABB	BBC	B	
56) 121971	F	26	A	AAB	B	A	AAB	—	BBD	ABB	ABB	BBC	A	
57) 121972	F	26	A	AAB	B	A	AAB	—	ABD	ABB	ABB	BBC	A	

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh	AcPh-2	Acon-1	Ck-1	Ck-2	Est-1	Got-1	Got-2	Idh-1	Ldb-1	Ldh-2	
			2	2	2	1	1	1	3	2	1	1	1	1
58)	134466	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
59)	134467	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
60)	134468	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
61)	134469	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
62)	134470	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
63)	134471	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
64)	134472	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
65)	134473	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
66)	134474	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
67)	134475	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
68)	134476	F	36	A	A	—	A	A	—	BBD	ABB	ABB	BBC	B
69)	134477	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
70)	134478	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
71)	134479	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
72)	134480	F	36	A	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
73)	134481	F	36	AAC	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
74)	135603	F	36	A	A	—	A	—	—	BBD	ABB	ABB	BBC	B
75)	139376	F	36	A	A	BCC	A	AAB	—	BBD	ABB	ABB	BCC	ABB
76)	139377	F	36	A	A	BCC	A	AAB	—	BBD	ABB	B	BBC	ABB
77)	139378	F	36	A	A	BCC	A	AAB	—	BBD	ABB	ABB	BBC	B
78)	139379	F	36	A	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B
79)	139380	F	36	A	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B
80)	139381	F	36	A	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B
81)	139382	F	36	A	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B
82)	122023	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
83)	122020	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	BBC	—
84)	122029	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
85)	122030	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
86)	122031	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
87)	122032	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
88)	122022	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
89)	122025	M	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
90)	122026	J	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
91)	122027	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
92)	122028	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
93)	122010	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
94)	122018	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
95)	122019	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
96)	122024	F	40	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	—
97)	108067	F	42	A	—	—	A	AAB	A	BBD	ABB	ABB	B	A
98)	122038	?	42	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	AAB
99)	122035	?	42	A	—	—	A	AAB	A	BBD	ABB	ABB	B	A
100)	122036	F	42	A	—	—	A	AAB	A	BBD	ABB	ABB	B	A
101)	122037	F	42	A	—	—	A	AAB	AAB	BBD	ABB	ABB	B	A
102)	122046	F	42	A	A	B	A	AAB	ABB	BBD	ABB	ABB	B	AAB
103)	122043	F	42	A	AAB	B	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB
104)	122045	F	42	A	A	B	A	AAB	A	BBD	ABB	ABB	BBC	A
105)	122041	F	42	A	—	—	A	AAB	AAB	BBD	ABB	ABB	BBC	A
106)	122044	F	42	A	AAB	B	A	AAB	A	BBD	ABB	ABB	BBC	A
107)	122039	F	42	A	AAB	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB
108)	122040	F	42	A	AAB	BBC	A	AAB	A	BBD	ABB	ABB	BBC	A
109)	108274	F	45	A	—	—	A	AAB	AAB	BBD	ABB	ABB	BBC	B
110)	108275	F	45	A	—	—	A	AAB	A	BBD	ABB	ABB	BBC	B
111)	114072	F	45	A	—	—	A	AAB	A	BBD	ABB	ABB	BBC	B
112)	114073	F	45	A	—	—	A	AAB	AAB	BBD	ABB	—	BBC	B
113)	114074	F	45	A	—	—	A	AAB	AAB	BBD	ABB	ABB	BBC	B
114)	114075	F	45	A	—	—	A	AAB	AAB	BBD	ABB	ABB	BBC	B

**APPENDIX B-4**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Ck-1 1	Ck-2 1	Est-1 1	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
115) 114076	F	45	A	—	—	A	AAB	A	BBD	ABB	ABB	BBC	B	
116) 114077	F	45	A	—	—	A	AAB	A	BBD	ABB	ABB	BBC	B	
117) 114078	F	45	A	—	—	A	AAB	A	BBD	ABB	ABB	BBC	B	
118) 114079	F	45	A	—	—	A	AAB	AAB	BBD	ABB	ABB	BBC	B	
119) 131655	F	45	A	—	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
120) 131656	F	45	A	AB	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	B	
121) 131657	F	45	A	AAB	B	A	AAB	AAB	BBD	ABB	ABB	BBC	B	
122) 131658	F	45	AAC	A	BBC	A	AAB	A	BBD	ABB	ABB	BBC	B	
123) 131659	F	45	AAC	AAB	BBC	A	AAB	A	BBD	ABB	ABB	BBC	B	
124) 131660	F	45	AAC	AAB	C	A	AAB	A	BBD	ABB	ABB	BBC	B	
125) 131661	F	45	A	AAB	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	B	
126) 131662	F	45	A	AAB	BBC	A	AAB	AAB	BBD	ABB	ABB	BBC	B	
127) 134486	F	48	A	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
128) 134487	F	48	A	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
129) 134488	F	49	A	ABB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
130) 134489	F	49	A	ABB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
131) 134490	F	49	A	ABB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
132) 122071	F	50	A	AAB	—	A	B	A	BBD	ABB	ABB	BBC	B	
133) 122075	F	50	A	AAB	—	A	B	AAB	BBD	ABB	ABB	BCC	B	
134) 122063	F	50	A	AAB	—	A	AAB	A	BBD	ABB	ABB	BCC	B	
135) 122072	F	50	A	AAB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	ABB	
136) 122074	F	50	A	AAB	—	A	ABB	AAB	BBD	ABB	ABB	BBC	B	
137) 122073	F	50	AAC	AAB	BCC	A	ABB	AAB	BBD	ABB	ABB	BBC	B	
138) 122076	F	58	A	AAB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	A	
139) 122077	F	58	A	AAB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	A	
140) 129433	F	58	A	A	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	
141) 129434	F	58	A	A	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	
142) 129435	F	58	A	A	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	
143) 114081	F	59	—	—	—	A	—	AAB	BBD	ABB	A	BBC	A	
144) 122080	F	62	—	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	A	
145) 122081	F	62	—	AAB	BCC	A	AAB	—	BBD	ABB	B	BBC	A	
146) 122082	F	62	—	AAB	BCC	A	AAB	—	BBD	ABB	B	BBC	AAB	
147) 122083	F	62	—	AAB	BCC	A	A	—	BBD	ABB	ABB	BBC	AAB	
148) 122084	F	62	—	—	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
149) 122085	F	62	—	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
150) 134514	F	62	—	—	B	A	AAB	—	BBD	ABB	B	BBC	AAB	
151) MCZ 107957	F	62	—	—	—	A	A	—	BBD	ABB	B	BBC	AAB	
152) MCZ 107958	F	62	—	—	B	A	AAB	—	BBD	ABB	B	BBC	AAB	
153) 131630	F	62	—	—	BCC	A	AAB	—	BBD	ABB	AAB	BBC	A	
154) 131632	F	62	AAC	AAB	BCC	A	AAB	AAB	BBD	ABB	ABB	BCC	B	
155) 131633	F	62	AAC	A	BBC	A	AAB	AAB	BBD	ABB	ABB	BCC	B	
156) 131634	F	62	ACC	A	BBC	A	AAB	AAB	BBD	ABB	A	BBC	ABB	
157) 134516	F	62	A	B	B	A	ABB	—	BBD	ABB	ABB	BBC	B	
158) 134517	F	62	AAC	A	BCC	A	ABB	—	BBD	ABB	ABB	BBC	B	
159) 129354	F	64	AAC	—	BCC	A	AAB	—	BBD	ABB	ABB	BBC	B	
160) 131646	F	64	ACC	AAB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
161) 131647	F	64	C	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
162) 131648	F	64	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	A	
163) 131649	F	64	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	A	
164) 131650	F	64	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
165) 131651	F	64	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
166) 131652	F	64	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
167) 129360	F	67	AAC	ABB	—	A	AAB	AAB	B	ABB	ABB	BBC	AAB	
168) 129361	F	67	AAC	ABB	—	A	ABB	AAB	BBD	ABB	ABB	BBC	B	
169) 129362	F	67	AAC	ABB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	
170) 129363	F	67	AAC	AAB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	
171) 129364	F	67	AAC	ABB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB	

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh	AcPh-2	Acon-1	Ck-1	Ck-2	Est-1	Got-1	Got-2	Idh-1	Ldh-1	Ldh-2	
			2	2	2	1	1	1	3	2	1	1	1	1
172)	129365	F	67	AAC	AAB	—	A	ABB	AAB	BBD	ABB	ABB	BBC	AAB
173)	129366	F	67	AAC	ABB	—	A	AAB	AAB	B	ABB	ABB	BBC	AAB
174)	129367	F	67	AAC	AAB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB
175)	129369	F	69	A	—	—	A	—	—	BBD	ABB	B	B	A
176)	129370	F	69	A	—	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB
177)	129371	F	69	A	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	A
178)	129395	F	70	AAC	—	B	A	AAB	—	BBD	ABB	AAB	BBC	AAB
179)	129396	F	70	ACC	—	BBC	A	ABB	—	BBD	ABB	ABB	BBC	AAB
180)	129397	F	70	C	—	B	A	AAB	—	BBD	ABB	ABB	BBC	AAB
181)	129398	F	70	A	—	BBC	A	AAB	—	BBD	ABB	ABB	BBC	A
182)	UMMZ 176840	F	74	—	—	—	A	—	—	BBD	ABB	ABB	B	AAB
183)	UMMZ 176839	F	74	—	—	—	A	—	—	BBD	ABB	ABB	BBC	A
184)	MCZ 107620	F	75	—	—	—	A	—	B	BBD	ABB	ABB	BBC	A
185)	MCZ 107622	F	75	—	—	—	A	AAB	B	BBD	ABB	B	B	A
186)	MCZ 107624	F	75	—	—	—	A	A	B	BBD	ABB	ABB	BBC	A
187)	MCZ 107626	F	75	—	—	—	A	—	ABB	BBD	ABB	ABB	BBC	AAB
188)	MCZ 107627	F	75	—	—	—	A	—	—	0	ABB	ABB	BBC	—
189)	MCZ 107629	?	75	—	—	—	A	—	—	0	ABB	ABB	BBC	A
190)	MCZ 107630	?	75	—	—	—	A	—	—	BBD	ABB	ABB	BBC	—
191)	MCZ 107631	?	75	—	—	—	A	AAB	ABB	—	ABB	ABB	BBC	AAB
192)	MCZ 107952	F	75	—	—	—	A	—	—	BBD	ABB	ABB	BBC	A
193)	MCZ 107633	F	75	—	—	—	A	—	—	BBD	ABB	ABB	BBC	A
194)	129446	F	75	—	AAB	—	A	ABB	AAB	BBD	ABB	ABB	BBC	AAB
195)	129447	F	75	—	AAB	—	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB
196)	129451	F	76	AAC	A	B	A	AAB	AAB	BBD	ABB	ABB	BBC	AAB
197)	139453	F	77	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
198)	139454	F	77	—	A	—	A	—	—	BBD	AAB	AAB	BBC	AAB
199)	139455	F	77	—	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
200)	140500	F	77	—	B	—	A	AAB	—	BBD	ABB	ABB	BBC	B
201)	140501	F	77	A	ABB	—	A	—	—	BBD	ABB	ABB	BBC	B
202)	140494	F	79	A	ABB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
203)	140495	F	79	A	ABB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
204)	139421	F	80	—	ABB	—	A	ABB	—	BBD	ABB	ABB	B	A
205)	139422	F	80	—	A	—	A	ABB	—	BBD	ABB	B	BBC	B
206)	139423	M	80	—	ABB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
207)	139424	F	80	—	AAB	—	A	AAB	—	BBD	ABB	ABB	B	B
208)	139425	F	80	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
209)	139426	F	80	—	AAB	—	A	AAB	—	BBD	ABB	B	BBC	B
210)	139427	F	80	—	A	—	A	AAB	—	BBD	ABB	B	BBC	B
211)	139428	F	80	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
212)	139429	F	80	—	B	—	A	A	—	BBD	ABB	ABB	BBC	A
213)	139430	F	80	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B
214)	139431	F	80	—	A	—	A	—	—	BBD	ABB	ABB	BBC	ABB
215)	140502	F	81	A	A	—	A	—	—	BBD	ABB	ABB	BBC	B
216)	140503	F	81	A	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
217)	140504	F	81	A	ABC	—	A	—	—	BBD	ABB	ABB	BBC	B
218)	134526	F	82	A	ABB	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B
219)	134527	F	82	A	?	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B
220)	134528	F	82	AAC	?	BBC	A	ABB	—	BBD	ABB	ABB	BBC	B
221)	134529	F	82	A	ABB	BCC	A	ABB	—	BBD	ABB	ABB	BBC	B
222)	134530	F	82	A	ABC	B	A	ABB	—	BBD	ABB	ABB	BBC	B
223)	134539	F	83	—	ABB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB
224)	134540	F	83	—	ABB	—	A	AAB	—	BBD	B	ABB	BBC	AAB
225)	134545	F	84	—	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
226)	134546	F	84	—	ABB	B	A	AAB	—	BBD	ABB	ABB	BBC	B
227)	134547	F	84	—	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B
228)	134548	F	84	—	AAB	BBC	A	AAB	—	BBD	AAB	ABB	BBC	B

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Ck-1 1	Ck-2 1	Est-1 1	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
229) 134549	F	84	—	AAB	B	A	AAB	—	BBD	AAB	ABB	BBC	B	
230) 134550	F	84	—	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
231) 134542	F	85	—	AAB	B	A	AAB	—	BBD	AAB	ABB	BBC	AAB	
232) 134556	F	87	A	A	B	A	AAB	—	BBD	ABB	ABB	BBC	B	
233) 134557	F	87	A	A	B	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
234) 134558	F	87	A	—	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
235) 134559	F	87	A	A	BCC	A	AAB	—	BBD	AAB	ABB	BBC	ABB	
236) 134560	F	87	A	A	BCC	A	AAB	—	BBD	ABB	ABB	BBC	B	
237) 134561	F	87	A	A	BCC	A	ABB	—	BBD	ABB	ABB	BBC	B	
238) 134562	F	87	A	A	BCC	A	ABB	—	BBD	ABB	ABB	BBC	B	
239) 134563	F	87	A	A	BCC	A	ABB	—	BBD	ABB	ABB	BBC	B	
240) 134564	F	87	A	AAB	BCC	A	ABB	—	BBD	ABB	ABB	BBC	B	
241) 134565	F	87	A	A	B	A	ABB	—	BBD	ABB	ABB	BBC	B	
242) 134567	F	88	—	A	BCC	A	AAB	—	BBD	AAB	ABB	BBC	B	
243) 134568	F	88	—	A	BCC	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
244) 134569	F	88	—	A	B	A	AAB	—	BBD	AAB	ABB	BBC	B	
245) 134570	F	88	—	A	B	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
246) 134571	F	88	—	A	B	A	AAB	—	BBD	AAB	ABB	BBC	B	
247) 134572	F	88	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
248) 134573	F	88	—	A	BBC	A	AAB	—	BBD	ABB	ABB	BBC	B	
249) 139480	F	89	—	AAB	—	A	A	—	BBD	AAB	ABB	BBC	B	
250) 139482	M	89	—	AAB	—	A	AAB	—	BBD	AAB	ABB	BBC	ABB	
251) 139483	M	89	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
252) 139484	F	89	—	B	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
253) 139485	F	89	—	B	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
254) 139486	F	89	—	ABB	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
255) 139487	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
256) 139488	F	89	—	B	—	A	AAB	—	BBD	ABB	ABB	BBC	A	
257) 139489	F	89	—	B	—	A	AAB	—	BBD	AAB	ABB	BBC	ABB	
258) 139492	F	89	—	B	—	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
259) 139493	F	89	—	A	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
260) 139494	F	89	—	B	—	A	AAB	—	BBD	ABB	ABB	BBC	B	
261) 139495	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
262) 139496	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
263) 139497	F	89	—	ABB	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
264) 139498	F	89	—	A	—	A	AAB	—	BBD	AAB	AAB	BBC	B	
265) 139499	F	89	—	B	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
266) 139500	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
267) 139501	F	89	—	ABB	—	A	AAB	—	BBD	ABB	ABB	BCC	ABB	
268) 139502	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
269) 139503	F	89	—	B	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
270) 139504	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
271) 139490	F	89	—	A	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
272) 139491	F	89	—	AAB	—	A	AAB	—	BBD	AAB	ABB	BBC	B	
273) 129456	F	91	ACC	—	BBC	A	AAB	—	BBD	ABB	ABB	B	A	
274) 129457	F	91	A	—	B	A	AAB	—	BBD	ABB	ABB	BBC	A	
275) 129458	F	91	AAC	—	BBC	A	AAB	—	BBD	ABB	ABB	BBC	A	
276) 129459	F	91	A	—	B	A	AAB	—	BBD	ABB	ABB	BBC	A	
277) 129460	F	91	AAC	—	BBC	A	AAB	—	BBD	ABB	ABB	B	A	
278) MCZ 110571	F	92	C	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
279) MCZ 110572	F	92	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
280) MCZ 110575	F	94	—	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
281) MCZ 110577	F	94	—	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
282) MCZ 110578	F	94	—	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
283) MCZ 110580	F	94	—	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	A	
284) MCZ 110582	F	94	—	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
285) MCZ 110583	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number											
			Adh	AcPh-2	Acon-1	Ck-1	Ck-2	Est-1	Got-1	Got-2	Idh-1	Ldh-1	Ldh-2	
			2	2	2	1	1	1	3	2	1	1	1	
286) MCZ 110584	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
287) MCZ 110585	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	ABB	
288) MCZ 110586	F	95	AAC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
289) MCZ 110587	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	B	
290) MCZ 110588	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	A	
291) MCZ 110589	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
292) MCZ 110590	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
293) MCZ 110591	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BCC	AAB	
294) MCZ 110592	F	95	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BCC	AAB	
295) MCZ 110595	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
296) MCZ 110596	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
297) MCZ 110597	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
298) MCZ 110598	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
299) MCZ 110600	F	96	A	AAB	BCC	A	—	—	BBD	ABB	ABB	BBC	AAB	
300) MCZ 110603	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
301) MCZ 110607	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
302) MCZ 110608	F	96	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
303) MCZ 110615	F	97	AAC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
304) MCZ 110616	F	97	AAC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
305) MCZ 110617	F	97	AAC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
306) MCZ 110611	F	98	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
307) MCZ 110612	F	98	A	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	A	
308) MCZ 110569	F	100	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
309) MCZ 110570	F	100	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
310) MCZ 110558	F	101	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
311) MCZ 110559	F	101	ACC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
312) MCZ 110563	F	102	ACC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
313) MCZ 110565	F	102	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
314) MCZ 110566	F	102	ACC	AAB	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
315) MCZ 110560	F	103	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
316) MCZ 110567	F	104	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	A	
317) MCZ 110568	F	104	ACC	AAC	BCC	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
318) MCZ 110561	F	105	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	
319) MCZ 110614	F	106	ACC	AAB	—	A	AAB	—	BBD	ABB	ABB	BBC	AAB	

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1	Mdh-2	Mdh-3	Mo-1	Mpi	Pgi	Pgm-1	Pgm-2	Sod-1	Sod-2	
			1	1	2	1	1	2	1	1	2	2	
1) 129237	F	1	BDD	B	B	AAB	ABB	—	BBC	B	BBD	976	
2) 129267	F	3	BDD	B	B	ABB	ABB	—	B	B	BBD	908	
3) 129263	F	3	BDD	B	B	ABB	ABB	—	B	B	BBD	950	
4) 129268	F	3	BDD	B	B	A	ABB	—	—	B	BBD	1044	
5) 129266	F	3	BDD	B	B	ABB	ABB	—	BBC	AAB	BBD	907	
6) 129269	F	3	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	875	
7) 129270	F	3	BDD	B	B	ABB	ABB	—	B	B	BBD	895	
8) 129271	F	3	BDD	B	B	AAB	ABB	—	—	B	BBD	1068	
9) 129272	F	3	BDD	B	B	ABB	ABB	ACC	B	B	BBD	850	
10) 129273	F	3	BDD	B	B	AAB	ABB	—	—	B	BBD	955	
11) 129274	F	3	BDD	B	B	AAB	ABB	—	—	B	BBD	954	
12) 129275	F	3	BDD	B	B	ABB	ABB	—	—	B	BBD	936	
13) 129264	F	3	BDD	B	B	AAB	ABB	—	BBC	B	BBD	1032	
14) 129265	F	3	BDD	B	B	AAB	ABB	ACC	BBC	B	BBD	897	
15) 129285	F	7	BDD	B	B	—	ABB	A	BBC	B	BBD	1022	
16) 129286	F	7	BDD	B	B	—	ABB	A	BBC	B	BBD	870	
17) 131600	F	15	BDD	B	B	ABB	ABB	ABB	B	B	BBD	1171	
18) 122117	F	16	BDD	B	B	AAB	—	—	B	BBD	906		

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Mdh-3 2	Me-1 1	Mpi 1	Pgi 2	Pgm-1 1	Pgm-2 1	Sod-1 2		
19)	122118	F	16	BDD	B	B	AAB	—	—	—	B	BBB	930
20)	131608	F	17	BDD	B	B	ABB	ABB	AAB	B	B	BBB	900
21)	131609	F	17	BDD	B	B	ABB	ABB	ABB	B	B	BBB	1059
22)	131610	F	17	BDD	B	B	AAB	ABB	ACC	B	B	BBB	1154
23)	131611	F	17	BDD	B	B	AAB	ABB	ABB	B	B	BBB	?
24)	131612	F	17	BDD	B	B	ABB	ABB	ACC	B	B	BBB	1108
25)	131613	M	17	BDD	B	B	ABB	ABB	ABB	BBC	B	BBB	1240
26)	135600	F	18	BDD	B	B	ABB	—	ACC	B	B	BBB	986
27)	140443	F	18	BDD	B	B	AAB	—	ACC	B	B	BBB	1075
28)	140444	F	18	BBD	B	B	AAB	—	AAC	BBC	B	BBB	762
29)	145035	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	895
30)	145037	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1133
31)	145038	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1155
32)	145039	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1289
33)	145040	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1088
34)	145041	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1104
35)	145043	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	868
36)	145044	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1092
37)	145045	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1010
38)	145046	F	18	BDD	B	B	AAB	ABB	ACC	B	B	BBB	976
39)	145050	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	1024
40)	145053	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	963
41)	145055	F	18	BDD	B	B	AAB	AAB	ACC	B	B	BBB	761
42)	145059	F	18	BDD	B	B	AAB	ABB	ACC	B	B	BBB	766
43)	121977	F	23	BBD	B	B	B	—	—	—	B	BBB	—
44)	121979	F	23	BBD	B	B	B	—	—	—	B	BBB	1119
45)	121980	F	23	BBD	B	B	B	—	—	—	B	BBB	897
46)	121978	F	23	BBD	B	B	ABB	—	—	B	B	BBB	1153
47)	121974	F	23	BBD	B	B	—	—	—	—	B	BBB	744
48)	121975	F	23	BBD	B	B	—	—	—	—	B	BBB	941
49)	121976	F	23	BBD	B	B	—	—	—	—	B	BBB	958
50)	121981	F	23	BBD	B	B	A	—	—	BBC	B	BBB	863
51)	121982	F	23	BBD	B	B	B	—	—	B	B	BBB	994
52)	121983	F	23	BDD	B	B	ABB	—	—	BCC	B	BBB	1046
53)	121984	F	23	BBD	B	B	ABB	—	—	B	B	BBB	1000
54)	121987	F	23	BBD	B	B	ABB	—	—	B	B	BBB	972
55)	121989	F	23	BBD	B	B	A	—	—	BBC	B	BBB	918
56)	121971	F	26	BDD	B	B	A	—	—	—	B	BBB	909
57)	121972	F	26	BBD	B	B	A	—	—	—	B	BBB	899
58)	134466	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	1069
59)	134467	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	1067
60)	134468	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	1096
61)	134469	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	968
62)	134470	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	1023
63)	134471	F	36	BDD	B	B	A	—	ACC	—	B	BBB	923
64)	134472	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	965
65)	134473	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	1014
66)	134474	F	36	BDD	B	B	ABB	—	ABC	—	B	BBB	?
67)	134475	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	910
68)	134476	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	1178
69)	134477	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	954
70)	134478	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	874
71)	134479	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	904
72)	134480	F	36	BDD	BBC	B	AAB	—	ACC	—	B	BBB	950
73)	134481	F	36	BDD	B	B	AAB	—	ACC	—	B	BBB	911
74)	135603	F	36	BDD	B	B	ABB	—	AAC	—	B	BBB	1125
75)	139376	F	36	BDD	B	B	ABB	ABB	—	B	B	BBB	1082

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Mdh-3 2	Me-1 1	Mpi 1	Pgi 2	Pgm-1 1	Pgm-2 1	Sod-1 2		
76) 139377	F	36	BDD	B	B	ABB	ABB	—	B	B	BBD	1118	
77) 139378	F	36	BDD	B	B	ABB	ABB	ACC	B	B	BBD	942	
78) 139379	F	36	BDD	B	B	ABB	ABB	ACC	B	B	BBD	1171	
79) 139380	F	36	BDD	B	B	ABB	ABB	ACC	B	B	BBD	1063	
80) 139381	F	36	BDD	B	B	ABB	ABB	ACC	B	B	BBD	958	
81) 139382	F	36	BDD	O	B	ABB	ABB	ACC	B	B	BBD	928	
82) 122023	F	40	BDD	B	B	B	—	—	—	B	BBD	1009	
83) 122020	F	40	BDD	B	B	B	—	—	—	B	BBD	912	
84) 122029	F	40	BDD	B	B	B	AAB	—	—	B	BBD	928	
85) 122030	F	40	BDD	B	B	B	—	—	—	B	BBD	889	
86) 122031	F	40	BDD	B	B	B	A	—	—	B	BBD	882	
87) 122032	F	40	BDD	B	B	B	A	—	—	B	BBD	960	
88) 122022	F	40	BDD	B	B	B	A	—	—	B	BBD	1003	
89) 122025	M	40	BDD	B	B	B	—	—	—	B	BBD	—	
90) 122026	J	40	BDD	B	B	B	—	—	—	B	BBD	882	
91) 122027	F	40	BDD	B	B	B	—	—	—	B	BBD	754	
92) 122028	F	40	BDD	B	B	B	—	—	—	B	BBD	—	
93) 122010	F	40	BDD	B	B	B	—	—	—	B	BBD	994	
94) 122018	F	40	BDD	B	ABB	B	—	—	—	B	BBD	1000	
95) 122019	F	40	BDD	B	ABB	B	—	—	—	B	BBD	1025	
96) 122024	F	40	BDD	B	B	B	—	—	—	B	BBD	1023	
97) 108067	F	42	BDD	B	B	B	—	—	B	B	BBD	—	
98) 122038	?	42	BDD	B	B	B	—	—	B	B	BBD	846	
99) 122035	?	42	BDD	B	B	B	—	—	B	B	BBD	834	
100) 122036	F	42	BDD	B	B	B	—	—	B	B	BBD	921	
101) 122037	F	42	BDD	B	B	B	—	—	B	B	BBD	1051	
102) 122046	F	42	BDD	B	B	B	—	—	B	B	BBD	865	
103) 122043	F	42	BDD	B	B	B	—	—	B	B	BBD	923	
104) 122045	F	42	BDD	B	B	B	—	—	B	B	BBD	1035	
105) 122041	F	42	BDD	B	B	B	—	—	B	B	BBD	873	
106) 122044	F	42	BDD	BBC	B	B	—	—	B	B	BBD	974	
107) 122039	F	42	BDD	BBC	B	B	—	—	B	B	BBD	896	
108) 122040	F	42	BDD	B	B	ABB	—	—	BBC	B	BBD	915	
109) 108274	F	45	BDD	B	B	—	—	—	B	B	BBD	947	
110) 108275	F	45	BDD	B	B	—	—	—	B	B	BBD	1001	
111) 114072	F	45	BDD	B	B	—	—	—	B	B	BBD	1060	
112) 114073	F	45	BDD	B	B	—	—	—	B	B	BBD	998	
113) 114074	F	45	BDD	B	B	—	—	—	B	B	BBD	984	
114) 114075	F	45	BDD	B	B	—	—	—	B	B	BBD	1171	
115) 114076	F	45	BDD	B	B	—	—	—	B	B	BBD	1008	
116) 114077	F	45	BDD	B	B	—	—	—	B	B	BBD	1032	
117) 114078	F	45	BDD	B	B	—	—	—	B	B	BBD	977	
118) 114079	F	45	BDD	B	B	—	—	—	B	B	BBD	723	
119) 131655	F	45	BDD	B	B	—	ABB	ACC	B	B	BBD	1022	
120) 131656	F	45	BDD	B	B	AAB	ABB	ACC	B	B	BBD	1143	
121) 131657	F	45	BDD	B	B	AAB	ABB	ACC	B	B	BBD	1086	
122) 131658	F	45	BDD	B	B	ABB	ABB	ACC	B	B	BBD	1006	
123) 131659	F	45	BDD	B	B	AAB	ABB	ACC	B	B	BBD	972	
124) 131660	F	45	BDD	B	B	AAB	ABB	ACC	B	B	BBD	1052	
125) 131661	F	45	BDD	B	B	ABB	ABB	ACC	B	B	BBD	1268	
126) 131662	F	45	BDD	B	B	ABB	ABB	ACC	B	B	BBD	1071	
127) 134486	F	48	BDD	B	B	A	ABB	ACC	BBC	B	BBD	894	
128) 134487	F	48	BDD	B	B	AAB	ABB	ACC	BBC	B	BBD	978	
129) 134488	F	49	BDD	B	B	AAB	ABB	ACC	BBC	B	BBD	1463	
130) 134489	F	49	BDD	B	B	AAB	—	ACC	BBC	B	BBD	1031	
131) 134490	F	49	BDD	B	B	AAB	—	ACC	BBC	B	BBD	861	
132) 122071	F	50	BDD	B	B	ABB	—	C	—	B	BBD	1027	

**APPENDIX B-4**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Mdh-3 2	Me-1 1	Mpi 1	Pgi 2	Pgm-1 1	Pgm-2 1	Sod-1 2		
133)	122075	F	50	BDD	BBC	B	ABB	ABB	C	—	B	BBB	1010
134)	122063	F	50	BDD	B	B	ABB	ABB	C	—	B	BBB	789
135)	122072	F	50	BDD	B	B	ABB	ABB	C	—	B	BBB	809
136)	122074	F	50	BDD	B	B	ABB	—	C	—	B	BBB	869
137)	122073	F	50	BDD	B	B	ABB	—	C	—	B	BBB	749
138)	122076	F	58	BDD	B	B	A	A	ACC	—	B	BBB	813
139)	122077	F	58	BDD	B	B	A	A	ACC	—	B	BBB	940
140)	129433	F	58	BDD	B	B	AAB	A	ACC	—	B	BBB	900
141)	129434	F	58	BDD	B	B	B	A	ACC	—	B	BBB	911
142)	129435	F	58	BDD	B	B	AAB	A	ACC	—	B	BBB	1153
143)	114081	F	59	BBD	B	B	B	—	—	B	B	BBB	—
144)	122080	F	62	BBD	B	B	—	—	ACC	B	B	BBB	885
145)	122081	F	62	BBD	B	B	—	—	ACC	B	B	BBB	776
146)	122082	F	62	BBD	B	B	—	—	ACC	B	B	BBB	771
147)	122083	F	62	BDD	B	ABB	—	—	ACC	B	B	BBB	810
148)	122084	F	62	BDD	B	B	—	—	ACC	BBC	B	BBB	803
149)	122085	F	62	BDD	B	ABB	—	—	AAC	B	B	BBB	772
150)	134514	F	62	BBD	B	B	—	—	ACC	B	B	BBB	721
151)	MCZ 107957	F	62	BDD	B	B	—	—	ACC	B	B	—	829
152)	MCZ 107958	F	62	BDD	B	B	—	—	ACC	B	B	BBB	815
153)	131630	F	62	BDD	B	B	—	—	ACC	B	B	BBB	965
154)	131632	F	62	BBD	B	B	AAB	ABB	ACC	B	B	BBB	—
155)	131633	F	62	BDD	B	B	AAB	ABB	ACC	B	B	BBB	1292
156)	131634	F	62	BDD	B	B	AAB	ABB	ACC	B	B	BBB	993
157)	134516	F	62	BDD	B	B	—	—	ACC	B	B	BBB	1184
158)	134517	F	62	BBD	B	B	AAB	—	AAC	B	B	BBB	1231
159)	129354	F	64	BDD	B	B	AAB	ABB	—	—	B	BBB	993
160)	131646	F	64	BDD	B	B	AAB	ABB	ACC	B	B	BBB	—
161)	131647	F	64	BDD	B	B	—	ABB	—	B	B	BBB	880
162)	131648	F	64	BDD	B	B	—	ABB	ACC	B	B	BBB	1116
163)	131649	F	64	BDD	B	B	—	ABB	ACC	B	B	BBB	991
164)	131650	F	64	BDD	B	B	—	ABB	ACC	B	B	BBB	1380
165)	131651	F	64	BDD	B	B	—	ABB	ACC	B	B	BBB	1186
166)	131652	F	64	BDD	B	B	—	ABB	ACC	B	B	BBB	1007
167)	129360	F	67	BDD	B	B	A	—	—	B	B	BBB	1004
168)	129361	F	67	BDD	B	B	A	—	—	B	B	BBB	985
169)	129362	F	67	BDD	B	B	A	—	—	B	B	BBB	947
170)	129363	F	67	BDD	B	B	A	—	—	B	B	BBB	866
171)	129364	F	67	BDD	B	B	AAB	—	—	BBC	B	BBB	830
172)	129365	F	67	BDD	B	B	AAB	—	—	BBC	B	BBB	850
173)	129366	F	67	BDD	B	B	A	—	—	B	B	BBB	926
174)	129367	F	67	BDD	B	B	A	—	—	B	B	BBB	871
175)	129369	F	69	BBC	B	B	A	ABB	—	—	B	BBB	—
176)	129370	F	69	BCC	B	B	AAB	ABB	—	BBC	B	BBB	735
177)	129371	F	69	BCC	B	B	AAB	ABB	—	?	B	BBB	917
178)	129395	F	70	BDD	B	B	AAB	ABB	ACC	BBC	B	BBB	788
179)	129396	F	70	BDD	B	B	AAB	ABB	AAC	B	B	BBB	938
180)	129397	F	70	BDD	B	B	AAB	ABB	ACC	BBC	B	BBB	862
181)	129398	F	70	BDD	B	B	AAB	ABB	ACC	B	B	BBB	682
182)	UMMZ 176840	F	74	BDD	B	B	—	AAB	—	B	B	BBB	—
183)	UMMZ 176839	F	74	BDD	B	B	—	—	—	B	B	BBB	874
184)	MCZ 107620	F	75	BDD	B	B	A	—	—	BBC	B	BBB	824
185)	MCZ 107622	F	75	BDD	B	B	A	—	—	BBC	B	BBB	875
186)	MCZ 107624	F	75	BDD	B	B	A	—	—	BBC	B	BBB	910
187)	MCZ 107626	F	75	BDD	B	B	ABB	—	—	—	B	BBB	—
188)	MCZ 107627	F	75	BDD	B	B	ABB	—	—	—	B	BBB	—
189)	MCZ 107629	?	75	BBD	B	B	A	—	—	—	B	0	—

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Mdh-3 2	Me-1 1	Mpi 1	Pgi 2	Pgm-1 1	Pgm-2 1	Sod-1 2		
190) MCZ 107630	?	75	—	B	B	A	—	—	—	B	0	—	—
191) MCZ 107631	?	75	BBD	B	B	A	—	—	BBC	B	BBD	—	—
192) MCZ 107952	F	75	BBD	B	B	ABB	—	—	—	B	BBD	1017	—
193) MCZ 107633	F	75	BDD	B	B	ABB	—	—	—	B	BBD	—	—
194) 129446	F	75	BDD	B	B	ABB	—	—	BBC	B	BBD	884	—
195) 129447	F	75	BDD	B	B	—	—	—	B	B	BBD	1020	—
196) 129451	F	76	BDD	B	B	B	—	—	B	B	BBD	908	—
197) 139453	F	77	BDD	B	B	ABB	—	ACD	BBC	B	BBD	796	—
198) 139454	F	77	BDD	B	B	A	—	AAC	B	B	BBD	1160	—
199) 139455	F	77	BDD	B	B	ABB	—	ACC	B	B	BBD	959	—
200) 140500	F	77	BDD	B	B	—	—	—	B	BBC	BBD	1028	—
201) 140501	F	77	BDD	B	B	B	—	AAC	B	B	BBD	1180	—
202) 140494	F	79	BDD	B	B	—	ABB	—	B	B	BBD	1041	—
203) 140495	F	79	BDD	B	B	—	ABB	—	B	B	BBD	1093	—
204) 139421	F	80	BDD	B	B	—	ABB	ACC	B	B	BBD	881	—
205) 139422	F	80	BDD	B	B	—	ABB	AAC	B	B	BBD	750	—
206) 139423	M	80	BDD	B	B	—	ABB	—	B	B	BBD	1167	—
207) 139424	F	80	BDD	B	B	A	—	ACC	B	B	BBD	1120	—
208) 139425	F	80	BDD	B	B	AAB	—	ACC	B	B	BBD	1119	—
209) 139426	F	80	BDD	B	B	A	—	AAC	B	B	BBD	1071	—
210) 139427	F	80	BDD	B	B	A	—	AAC	B	B	BBD	1084	—
211) 139428	F	80	BDD	B	B	AAB	—	ACC	B	B	BBD	978	—
212) 139429	F	80	BDD	B	B	A	—	—	B	B	BBD	962	—
213) 139430	F	80	BDD	B	B	AAB	—	ACC	B	B	BBD	1021	—
214) 139431	F	80	BDD	B	B	ABB	—	AAC	B	B	BBD	977	—
215) 140502	F	81	BDD	B	B	AAB	ABB	AAC	B	B	BBD	1292	—
216) 140503	F	81	BDD	B	B	—	ABB	BCC	B	B	BBD	1269	—
217) 140504	F	81	BDD	B	B	AAB	—	AAC	B	B	BBD	1182	—
218) 134526	F	82	BDD	B	B	AAB	ABB	AAC	B	B	BBD	1081	—
219) 134527	F	82	BDD	B	B	ABB	ABB	AAC	B	B	BBD	1131	—
220) 134528	F	82	BDD	B	B	—	—	ACC	B	B	BBD	1216	—
221) 134529	F	82	BDD	B	B	AAB	—	AAC	B	B	BBD	1090	—
222) 134530	F	82	BDD	B	B	—	—	AAC	B	B	BBD	1140	—
223) 134539	F	83	BDD	B	B	ABB	—	ACC	B	B	BBD	1077	—
224) 134540	F	83	BDD	B	B	ABB	—	ACC	B	B	BBD	1450	—
225) 134545	F	84	BDD	B	B	ABB	—	ACC	B	B	BBD	1203	—
226) 134546	F	84	BDD	B	B	AAB	—	ACC	B	B	BBD	980	—
227) 134547	F	84	BDD	B	B	ABB	—	ACC	B	B	BBD	1148	—
228) 134548	F	84	BDD	B	B	AAB	—	ACC	B	B	BBD	1063	—
229) 134549	F	84	BDD	B	B	AAB	—	ACC	B	B	BBD	1052	—
230) 134550	F	84	BDD	B	B	?	—	ACC	B	B	BBD	887	—
231) 134542	F	85	BDD	B	B	ABB	—	ACC	B	B	D	1129	—
232) 134556	F	87	BDD	B	B	AAB	—	ACC	BBC	B	BBD	1094	—
233) 134557	F	87	BDD	B	B	A	—	ACC	BBC	B	BBD	1030	—
234) 134558	F	87	BDD	B	B	A	—	ACC	BBC	B	BBD	1123	—
235) 134559	F	87	BDD	B	B	ABB	—	ACC	BBC	B	BBD	1312	—
236) 134560	F	87	BDD	B	B	AAB	—	ACC	B	B	BBD	997	—
237) 134561	F	87	BDD	B	B	AAB	—	AAC	B	B	BBD	1072	—
238) 134562	F	87	BDD	B	B	AAB	—	AAC	B	B	BBD	1219	—
239) 134563	F	87	BDD	B	B	AAB	—	AAC	B	B	BBD	948	—
240) 134564	F	87	BDD	B	B	AAB	—	AAC	B	B	BBD	1072	—
241) 134565	F	87	BDD	B	B	ABB	—	ACC	BBC	B	BBD	1145	—
242) 134567	F	88	BDD	B	B	ABB	—	ACC	BBC	B	BBD	994	—
243) 134568	F	88	BDD	B	B	ABB	—	ACC	BBC	B	BBD	1132	—
244) 134569	F	88	BDD	B	B	A	—	ACC	BBC	B	BBD	1149	—
245) 134570	F	88	BDD	B	B	AAB	—	ACC	BBC	BBC	BBD	1174	—
246) 134571	F	88	BDD	B	B	AAB	—	ACC	BBC	B	BBD	1137	—

**APPENDIX B-4**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Mdh-3 2	Me-1 1	Mpi 1	Pgi 2	Pgm-1 1	Pgm-2 1	Sod-1 1	Sod-1 2	
247)	134572	F	88	BDD	B	B	AAB	—	AAC	BBC	B	BBD	1337
248)	134573	F	88	BDD	B	B	A	—	ACC	B	B	BBD	1280
249)	139480	F	89	BDD	B	B	ABB	—	ACC	B	B	BBD	939
250)	139482	M	89	BDD	B	B	ABB	—	—	BBC	B	BBD	948
251)	139483	M	89	BDD	B	B	A	—	AAC	BBC	B	BBD	982
252)	139484	F	89	BDD	B	B	—	ABB	—	BBC	B	BBD	1173
253)	139485	F	89	BDD	B	B	A	—	—	BBC	B	BBD	1087
254)	139486	F	89	BDD	B	B	—	ABB	—	BBC	B	BBD	981
255)	139487	F	89	BDD	B	B	A	—	ACC	BBC	B	BDD	1025
256)	139488	F	89	BDD	B	B	ABB	—	—	?	B	BBD	1034
257)	139489	F	89	BDD	B	B	A	—	—	BBC	B	BBD	969
258)	139492	F	89	BDD	B	B	A	—	—	?	B	BBD	1105
259)	139493	F	89	BDD	B	B	ABB	—	ACC	BBC	B	D	1117
260)	139494	F	89	BDD	B	B	A	—	AAC	BBC	B	BBD	1086
261)	139495	F	89	BDD	B	B	ABB	—	ACD	BBC	B	D	1005
262)	139496	F	89	BDD	B	B	ABB	—	ACC	B	B	BBD	1023
263)	139497	F	89	BDD	B	B	—	ABB	—	BBC	B	BBD	1181
264)	139498	F	89	BDD	B	B	ABB	—	AAC	BBC	B	BDD	996
265)	139499	F	89	BDD	B	B	A	—	—	BBC	B	BBD	1082
266)	139500	F	89	BDD	B	B	A	—	AAC	BBC	B	BBD	1042
267)	139501	F	89	BDD	B	B	ABB	—	AAC	B	B	BBD	1050
268)	139502	F	89	BDD	B	B	A	—	AAC	BBC	B	D	1007
269)	139503	F	89	BDD	B	B	AAB	—	AAC	BBC	B	BBD	1048
270)	139504	F	89	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1137
271)	139490	F	89	BDD	B	B	ABB	—	ACC	B	B	BBD	926
272)	139491	F	89	BDD	B	B	ABB	—	ACC	BBC	B	BBD	936
273)	129456	F	91	BDD	B	B	A	ABB	—	BBC	B	BBD	969
274)	129457	F	91	BDD	B	B	A	ABB	ACC	BBC	B	BBD	1020
275)	129458	F	91	BDD	B	B	A	B	—	BBC	B	BBD	1167
276)	129459	F	91	BDD	B	B	A	ABB	ACC	BBC	B	BBD	1007
277)	129460	F	91	BDD	B	B	A	B	—	BBC	B	BBD	934
278)	MCZ 110571	F	92	BDD	B	B	A	ABB	AAC	BBC	B	BBD	1106
279)	MCZ 110572	F	92	BDD	B	B	AAB	ABB	AAB	BBC	B	BBD	1019
280)	MCZ 110575	F	94	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1030
281)	MCZ 110577	F	94	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	922
282)	MCZ 110578	F	94	BDD	B	B	—	—	ACC	BBC	B	BBD	1065
283)	MCZ 110580	F	94	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1136
284)	MCZ 110582	F	94	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1150
285)	MCZ 110583	F	95	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1163
286)	MCZ 110584	F	95	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1158
287)	MCZ 110585	F	95	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1061
288)	MCZ 110586	F	95	BDD	B	B	—	A	ACC	BBC	B	BBD	1140
289)	MCZ 110587	F	95	BDD	B	B	—	ABB	AAC	BBC	B	BBD	1155
290)	MCZ 110588	F	95	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1224
291)	MCZ 110589	F	95	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1174
292)	MCZ 110590	F	95	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1217
293)	MCZ 110591	F	95	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1088
294)	MCZ 110592	F	95	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1122
295)	MCZ 110595	F	96	BDD	B	B	A	ABB	ACC	BBC	B	BBD	1100
296)	MCZ 110596	F	96	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1061
297)	MCZ 110597	F	96	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1086
298)	MCZ 110598	F	96	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1052
299)	MCZ 110600	F	96	BDD	B	B	ABB	ABB	—	BBC	B	BBD	1062
300)	MCZ 110603	F	96	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1161
301)	MCZ 110607	F	96	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1086
302)	MCZ 110608	F	96	BDD	B	B	ABB	ABB	ACC	BBC	B	BBD	1020
303)	MCZ 110615	F	97	BDD	B	B	—	ABB	ACC	BBC	B	BBD	1043

APPENDIX B-4  
(Continued)

Specimen	Sex	Site	Locus and gel number										Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-2 1	Mdh-3 2	Mo-1 1	Mpi 1	Pgi 2	Pgm-1 1	Pgm-2 1	Sod-1 2		
304) MCZ 110616	F	97	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1091	
305) MCZ 110617	F	97	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1113	
306) MCZ 110611	F	98	BDD	B	B	—	ABB	AAC	BBC	B	BBB	1161	
307) MCZ 110612	F	98	BDD	B	B	ABB	—	AAC	BBC	B	BBB	1018	
308) MCZ 110569	F	100	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1062	
309) MCZ 110570	F	100	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1082	
310) MCZ 110558	F	101	BDD	B	B	AAB	ABB	ACC	BBC	B	BBB	1058	
311) MCZ 110559	F	101	BDD	B	B	AAB	ABB	AAB	BBC	B	BBB	1036	
312) MCZ 110563	F	102	BDD	B	B	AAB	ABB	AAB	BBC	B	BBB	1106	
313) MCZ 110565	F	102	BDD	B	B	AAB	ABB	ACC	BBC	B	BBB	1125	
314) MCZ 110566	F	102	BDD	B	B	AAB	ABB	AAB	BBC	B	BBB	915	
315) MCZ 110560	F	103	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1029	
316) MCZ 110567	F	104	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1008	
317) MCZ 110568	F	104	BDD	B	B	—	ABB	ACC	BBC	B	BBB	1017	
318) MCZ 110561	F	105	BDD	B	B	AAB	ABB	ACC	BBC	B	BBB	998	
319) MCZ 110614	F	106	BDD	B	B	AAB	ABB	ACC	BCC	B	BBB	1124	

APPENDIX B-5  
*Ambystoma laterale*-(2) *jeffersonianum* Triploid Hybrids

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 1	Ck-1 1	Ck-2 1	Est-1 3	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
1) 129277	F	4	—	—	—	A	ABB	ABB	BDD	AAB	AAB	BCC	B	
2) 122136	F	5	A	AAB	B	A	—	—	BDD	AAB	AAB	BCC	B	
3) 122137	F	5	A	AAB	BBC	A	—	—	BDD	AAB	AAB	BCC	B	
4) 122138	F	5	A	AAB	BBC	A	—	—	BDD	AAB	AAB	BCC	B	
5) 122139	F	5	AAC	AAB	B	A	—	—	BDD	AAB	AAB	BCC	B	
6) 122140	F	5	AAC	AAB	B	A	—	—	BDD	AAB	AAB	BCC	B	
7) 122141	F	5	A	AAB	C	A	—	—	BDD	AAB	AAB	BCC	B	
8) 122148	F	5	A	AAB	B	A	—	—	BDD	AAB	AAB	BCC	ABB	
9) 122149	F	5	A	AAB	B	A	—	—	BDD	AAB	AAB	BCC	B	
10) 122150	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
11) 122151	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
12) 122152	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
13) 122153	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
14) 122154	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
15) 122155	F	5	AAC	AAB	B	A	—	—	BDD	AAB	AAB	BCC	B	
16) 122145	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	A	
17) 122146	F	5	A	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
18) 122142	F	5	A	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
19) 122143	F	5	AAC	AAB	BCC	A	—	—	BDD	AAB	AAB	BCC	B	
20) 122147	F	5	A	AAB	BBC	A	—	—	BDD	AAB	AAB	BCC	B	

**APPENDIX B-5**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 1	Ck-1 1	Ck-2 1	Est-1 3	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
21)	122144	F	5	A	AAB	BBC	A	—	—	BDD	AAB	AAB	BCC	B
22)	122120	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	A	BCC	B
23)	122133	F	6	—	B	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
24)	122126	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
25)	122127	F	6	—	ABB	BCC	AAB	ABB	—	BDD	AAB	AAB	BBC	B
26)	122125	F	6	—	ABB	BCC	A	AAB	—	BDD	AAB	AAB	BCC	B
27)	122124	F	6	—	ABB	B	A	ABB	—	BDD	AAB	AAB	BCC	B
28)	122130	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	ABB
29)	122128	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	ABB
30)	122129	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
31)	122131	F	6	—	ABB	C	A	ABB	—	BDD	AAB	AAB	BCC	B
32)	122121	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	ABB
33)	122122	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BBC	B
34)	122123	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
35)	122132	F	6	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
36)	122087	F	8	—	—	—	A	—	ABB	BDD	—	—	BCC	—
37)	139537	F	9	A	ABB	—	A	—	—	BDD	AAB	AAB	BCC	B
38)	122088	F	10	—	ABB	—	A	—	ABB	BDD	—	AAB	BCC	ABB
39)	122089	F	10	—	ABB	—	A	—	ABB	BDD	—	AAB	BCC	ABB
40)	131560	F	11	A	ABB	BCC	A	AAB	ABB	BDD	AAB	AAB	BBC	—
41)	131561	F	11	AAC	—	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	—
42)	131562	F	11	AAC	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	C	—
43)	131563	F	11	ACC	—	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	—
44)	131564	F	11	ACC	ABB	BCC	AAB	ABB	ABB	BDD	AAB	AAB	BCC	—
45)	131565	F	11	ACC	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	—
46)	131566	F	11	A	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	—
47)	131567	F	11	ACC	ABB	BCC	AAB	ABB	ABB	BDD	AAB	AAB	C	—
48)	131568	F	11	ACC	ABB	BCC	A	AAB	ABB	BDD	AAB	AAB	BCC	—
49)	122101	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
50)	122102	F	12	A	ABB	C	A	—	—	BDD	AAB	AAB	BCC	B
51)	122103	F	12	A	ABB	B	A	—	—	BDD	AAB	AAB	BCC	B
52)	122111	F	12	A	ABB	C	A	—	—	BDD	AAB	AAB	BCC	B
53)	122112	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	ABB
54)	122115	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
55)	122116	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
56)	122106	F	12	A	ABB	C	A	—	—	BDD	AAB	B	BCC	B
57)	122105	F	12	AAC	ABB	C	A	—	—	BDD	AAB	AAB	BCC	B
58)	122107	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
59)	122108	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
60)	122109	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
61)	122100	F	12	A	ABB	BCC	A	—	—	BDD	AAB	AAB	BCC	B
62)	122099	F	12	A	ABB	B	A	—	—	BDD	AAB	AAB	BBC	B
63)	122098	F	12	A	ABB	C	A	—	—	BDD	AAB	AAB	BCC	B
64)	122097	F	12	A	ABB	B	A	—	—	BDD	AAB	AAB	BCC	B
65)	131584	F	13	A	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	B
66)	131585	F	13	AAC	ABB	BCC	AAB	ABB	ABB	BDD	AAB	AAB	BCC	B
67)	131586	F	13	A	ABB	BCC	A	AAB	ABB	BDD	AAB	AAB	BCC	B
68)	131587	F	13	C	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
69)	131588	F	13	A	ABB	BCC	A	AAB	ABB	BDD	AAB	AAB	BCC	B
70)	131589	F	13	A	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	B
71)	131590	F	13	—	—	—	A	AAB	—	BBD	ABB	ABB	BBC	ABB
72)	131594	F	14	A	A	BCC	A	AAB	—	BDD	AAB	AAB	BCC	B
73)	131595	F	14	AAC	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	B
74)	131596	F	14	A	ABB	C	A	ABB	ABB	BDD	AAB	AAB	BBC	B
75)	131597	F	14	A	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	B
76)	131598	F	14	ACC	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	B
77)	131599	F	15	ACC	ABB	BCC	A	ABB	AAB	BDD	AAB	AAB	BCC	B

APPENDIX B-5  
(Continued)

Specimen	Sex	Size	Locus and gel number											
			Adh-2	AcPh-2	Acon-1	Ck-1	Ck-2	Est-1	Got-1	Got-2	Idh-1	Ldb-1	Ldh-2	
78)	122090	F	19	AAC	—	—	A	AAB	—	BDD	AAB	ABB	BBC	B
79)	122091	F	19	—	—	—	A	AAB	—	BDD	AAB	ABB	BBC	B
80)	122092	F	19	—	—	—	A	AAB	—	BDD	AAB	ABB	BBC	B
81)	122093	F	19	—	—	—	A	AAB	—	BDD	AAB	ABB	BBC	B
82)	122094	F	19	—	—	—	A	AAB	—	BDD	AAB	ABB	BBC	A
83)	122095	F	19	A	—	—	A	AAB	—	BDD	AAB	AAB	BCC	AAB
84)	122096	F	19	—	—	—	A	AAB	—	BDD	AAB	A	BBC	B
85)	129304	F	20	AAC	B	BCC	A	AAB	—	BDD	AAB	AAB	BCC	B
86)	129305	F	20	—	ABB	BCC	AAB	AAB	—	BDD	AAB	AAB	BCC	ABB
87)	129306	F	20	—	ABB	BCC	AAB	ABB	—	BDD	AAB	AAB	BCC	A
88)	129307	F	20	A	ABB	B	AAB	ABB	—	BDD	AAB	AAB	BCC	ABB
89)	129308	F	20	—	ABB	BCC	AAB	ABB	—	BDD	AAB	AAB	BCC	ABB
90)	129309	F	20	—	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	ABB
91)	129310	F	20	—	ABB	BCC	ABB	ABB	—	BDD	AAB	AAB	BCC	A
92)	134462	F	20	A	AAB	B	A	ABB	—	BDD	AAB	AAB	BCC	B
93)	135601	F	21	—	AAB	—	A	—	—	BDD	AAB	AAB	BCC	B
94)	129299	F	22	A	A	C	A	AAB	—	BDD	AAB	AAB	BCC	B
95)	129300	F	22	AAC	AAB	C	A	AAB	—	BDD	AAB	AAB	BCC	B
96)	129296	F	22	A	A	C	A	AAB	—	BDD	AAB	AAB	BCC	B
97)	129297	F	22	AAC	A	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
98)	129298	F	22	A	A	C	A	AAB	—	BDD	AAB	AAB	BCC	B
99)	129292	F	22	A	ABB	BBC	A	AAB	—	BDD	AAB	AAB	BCC	B
100)	129314	F	22	A	A	BBC	A	AAB	—	BDD	AAB	AAB	BCC	ABB
101)	121991	F	23	—	—	—	A	—	—	BDD	AAB	AAB	B	B
102)	114070	F	25	—	—	—	A	—	ABB	BDD	AAB	AAB	BCC	B
103)	114059	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
104)	114060	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
105)	114061	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
106)	114062	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	C	ABB
107)	114063	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
108)	114064	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
109)	121968	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	B	B
110)	121969	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
111)	121970	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
112)	129412	F	27	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
113)	114065	F	28	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
114)	114080	F	29	—	—	—	—	—	ABB	BDD	—	A	BCC	ABB
115)	129325	F	30	A	ABB	BCC	AAB	AAB	ABB	BDD	AAB	AAB	BCC	B
116)	129326	F	30	A	ABB	C	A	AAB	ABB	BDD	AAB	AAB	BCC	B
117)	129322	F	30	ACC	ABB	BCC	A	ABB	ABB	BDD	AAB	AAB	BCC	ABB
118)	129323	F	30	A	ABB	BCC	A	AAB	ABB	BDD	B	AAB	BCC	B
119)	129324	F	30	A	ABB	BCC	AAB	AAB	ABB	BDD	AAB	AAB	BCC	A
120)	129327	F	30	A	ABB	BCC	ABB	AAB	ABB	BDD	AAB	AAB	BCC	B
121)	129315	F	31	A	ABB	C	—	ABB	—	BDD	AAB	AAB	BCC	ABB
122)	134463	F	31	A	AAB	C	—	ABB	—	BDD	AAB	AAB	BCC	B
123)	134464	F	31	A	A	C	—	ABB	—	BDD	AAB	AAB	BCC	B
124)	134484	F	31	A	B	C	—	ABB	—	BDD	AAB	AAB	BCC	B
125)	131620	F	32	A	A	BCC	A	AAB	—	BDD	AAB	AAB	BCC	B
126)	131621	F	32	ACC	A	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
127)	131625	F	32	A	A	C	A	ABB	—	BDD	AAB	AAB	BCC	B
128)	131626	F	32	A	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	ABB
129)	131627	F	32	—	ABB	—	AAB	—	—	BDD	ABB	ABB	BBC	ABB
130)	131628	F	32	ACC	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
131)	131622	F	32	ACC	ABB	BCC	ABB	ABB	—	BDD	AAB	AAB	BCC	B
132)	131623	F	32	ACC	ABB	BCC	A	ABB	—	BDD	AAB	AAB	BCC	B
133)	131624	F	32	A	ABB	C	A	ABB	—	BDD	AAB	AAB	BCC	ABB
134)	122009	F	33	AAC	—	BCC	—	AAB	—	BDD	AAB	AAB	BCC	ABB

**APPENDIX B-5**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 1	Ck-1 1	Ck-2 1	Est-1 3	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
135)	114066	F	34	AAC	—	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
136)	129419	F	34	AAC	—	BCC	—	ABB	—	BDD	AAB	AAB	BBC	B
137)	129420	F	34	A	—	BCC	—	ABB	—	BDD	AAB	AAB	BCC	ABB
138)	134482	F	37	—	ABB	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
139)	135602	F	39	A	ABB	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
140)	129425	F	41	—	—	—	—	—	—	BDD	AAB	AAB	BCC	B
141)	129426	F	41	—	—	—	—	—	—	BDD	AAB	AAB	BCC	ABB
142)	129427	F	41	A	A	—	—	ABB	—	BDD	AAB	AAB	BCC	B
143)	129421	F	41	—	—	—	—	—	—	BDD	AAB	AAB	BCC	B
144)	129422	F	41	A	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
145)	129423	F	41	A	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
146)	129424	F	41	—	—	—	—	ABB	—	BDD	AAB	AAB	BBC	B
147)	135608	F	43	—	ABB	BCC	—	ABB	—	BDD	AAB	AAB	BBC	B
148)	135609	F	43	—	B	BCC	—	ABB	—	BDD	AAB	AAB	BBC	B
149)	135610	F	43	—	ABB	—	—	ABB	—	BDD	AAB	AAB	BCC	B
150)	135611	F	43	—	ABB	—	—	ABB	—	BDD	AAB	AAB	BCC	B
151)	135612	F	43	—	AAB	—	—	ABB	—	BDD	AAB	AAB	BCC	B
152)	135613	F	43	—	A	—	—	ABB	—	BDD	AAB	AAB	BCC	B
153)	135614	F	43	—	A	—	—	ABB	—	BDD	AAB	AAB	BCC	B
154)	135615	F	43	—	A	—	—	ABB	—	BDD	AAB	AAB	BBC	B
155)	135616	F	43	—	A	—	—	AAB	—	BDD	AAB	AAB	BBC	B
156)	135617	F	43	—	B	—	—	ABB	—	BDD	AAB	AAB	BBC	B
157)	135618	F	43	—	ABB	—	—	ABB	—	BDD	AAB	AAB	BCC	B
158)	122004	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
159)	122002	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
160)	122003	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
161)	122001	F	44	—	—	—	—	—	ABB	BDD	—	—	—	—
162)	122005	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
163)	122006	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
164)	122007	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
165)	122008	F	44	—	—	—	—	—	ABB	BDD	—	—	BCC	B
166)	108268	F	46	—	—	—	—	—	AAB	BDD	AAB	AAB	BCC	B
167)	108269	F	46	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
168)	108270	F	46	—	—	—	—	—	AAB	BDD	AAB	AAB	BCC	B
169)	108271	F	46	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
170)	108272	F	46	—	—	—	—	—	ABB	BDD	AAB	B	BCC	B
171)	108273	F	46	—	—	—	—	—	ABB	BDD	AAB	AAB	BCC	B
172)	108276	J	46	—	—	—	—	—	ABB	BDD	AAB	A	BCC	B
173)	108025	F	46	—	—	—	—	—	ABB	BDD	AAB	—	BCC	B
174)	114068	F	46	—	—	—	—	—	—	BDD	AAB	—	BCC	B
175)	114067	F	46	—	—	—	—	—	—	BDD	AAB	—	BCC	B
176)	114071	F	46	—	—	—	—	—	ABB	BDD	AAB	—	BCC	B
177)	114069	F	46	—	—	—	—	—	—	BDD	AAB	—	BCC	B
178)	124702	F	46	—	—	—	—	—	ABB	BDD	AAB	—	BCC	B
179)	131663	F	46	ACC	ABB	BCC	—	AAB	AAB	BDD	AAB	AAB	BCC	B
180)	131664	F	46	C	ABB	BCC	—	AAB	ABB	BDD	AAB	AAB	BCC	B
181)	131665	F	46	C	AAB	BCC	—	ABB	ABB	BDD	AAB	AAB	BCC	B
182)	134494	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
183)	134495	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
184)	134496	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
185)	134497	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
186)	134499	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
187)	134500	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
188)	134501	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
189)	134502	F	46	—	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
190)	139411	F	46	A	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
191)	139412	F	46	A	A	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B

**APPENDIX B-5**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 1	Ck-1 1	Ck-2 1	Est-1 3	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
192)	139386	F	47	A	A	BCC	—	—	—	BDD	AAB	AAB	BCC	B
193)	139387	F	51	A	—	—	A	ABB	—	BDD	AAB	AAB	BCC	B
194)	139388	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
195)	139389	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
196)	139390	F	51	A	A	—	A	ABB	—	BDD	AAB	A	BCC	B
197)	139391	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
198)	139392	F	51	—	—	—	—	—	—	—	—	—	—	B
199)	139393	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
200)	139394	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
201)	139395	F	51	A	A	—	A	ABB	—	BDD	AAB	AAB	BCC	B
202)	139396	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
203)	139397	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
204)	139398	F	51	A	A	—	A	AAB	—	BDD	AAB	AAB	BBC	B
205)	139399	F	51	A	A	—	A	ABB	—	BDD	AAB	A	BCC	B
206)	139400	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
207)	139401	F	51	A	A	—	A	ABB	—	BDD	AAB	AAB	BCC	B
208)	139402	F	51	A	A	—	A	ABB	—	BDD	AAB	AAB	BCC	B
209)	139403	F	51	A	AAB	—	A	AAB	—	BDD	AAB	AAB	BCC	B
210)	139404	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
211)	139405	F	51	A	A	—	A	ABB	—	BDD	AAB	AAB	BCC	B
212)	139406	F	51	A	A	—	A	ABB	—	BDD	AAB	AAB	BCC	B
213)	139407	F	51	A	ABB	—	A	AAB	—	BDD	AAB	AAB	BCC	B
214)	139408	F	51	A	AAB	—	—	—	—	BDD	AAB	—	—	B
215)	139409	F	51	A	A	—	—	—	—	BDD	AAB	—	—	B
216)	129335	F	52	A	B	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
217)	129336	F	52	—	ABB	B	—	ABB	—	BDD	AAB	AAB	BCC	B
218)	129337	F	52	—	ABB	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
219)	129338	F	52	A	B	BCC	—	ABB	—	BDD	AAB	A	BCC	B
220)	129339	F	52	A	B	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
221)	129340	F	52	—	B	BCC	—	ABB	—	BDD	AAB	AAB	BCC	B
222)	131638	F	54	AAC	ABB	—	AAB	ABB	—	BDD	AAB	AAB	BCC	B
223)	131639	F	54	AAC	ABB	—	AAB	ABB	—	BDD	AAB	AAB	BCC	B
224)	131640	F	54	AAC	ABB	—	AAB	ABB	—	BDD	AAB	AAB	BCC	B
225)	129344	F	55	—	ABB	BCC	—	—	—	BDD	AAB	AAB	BCC	B
226)	129345	F	55	—	A	BCC	—	—	—	BDD	AAB	AAB	BCC	B
227)	129346	F	55	—	A	BCC	—	—	—	BDD	AAB	AAB	BCC	B
228)	129347	F	56	ACC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
229)	129348	F	56	ACC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
230)	129349	F	56	ACC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
231)	129350	F	56	AAC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
232)	129351	F	56	AAC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
233)	129352	F	56	ACC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
234)	129353	F	56	ACC	—	BCC	—	—	—	BDD	AAB	AAB	BCC	B
235)	131641	F	56	ACC	AAB	BCC	ABB	ABB	—	BDD	AAB	AAB	BCC	B
236)	131642	F	56	C	AAB	BCC	AAB	ABB	—	BDD	AAB	AAB	BCC	B
237)	131643	F	56	C	AAB	BCC	AAB	ABB	—	BDD	AAB	AAB	BCC	B
238)	131644	F	56	—	AAB	BCC	ABB	ABB	—	BDD	AAB	AAB	BCC	B
239)	131645	F	56	ACC	AAB	BCC	ABB	ABB	—	BDD	AAB	AAB	BCC	B
240)	131635	F	63	A	ABB	BCC	—	ABB	ABB	BDD	AAB	AAB	BCC	B
241)	131636	F	63	A	ABB	BCC	—	ABB	ABB	BDD	AAB	AAB	BCC	B
242)	131637	F	63	A	ABB	BBC	—	ABB	ABB	BDD	AAB	AAB	BCC	B
243)	129454	F	72	ACC	B	BCC	—	AAB	—	BDD	AAB	AAB	BCC	ABB
244)	129452	F	76	A	ABB	B	—	ABB	A	BDD	AAB	AAB	BCC	ABB
245)	129453	F	76	ACC	ABB	B	—	AAB	ABB	BDD	AAB	AAB	BCC	ABB
246)	139441	F	76	—	ABC	B	—	AAB	—	BDD	ABB	AAB	BCC	B
247)	139442	F	76	—	ABC	B	—	—	—	BDD	AAB	AAB	BCC	B
248)	139443	F	76	—	ABC	BCC	—	ABB	—	BDD	AAB	AAB	BCC	ABB

**APPENDIX B-5**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 1	Ck-1 1	Ck-2 1	Est-1 3	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
249) 139444	F	76	—	ABC	B	—	—	—	BDD	AAB	AAB	BCC	B	
250) 139445	F	76	—	ABC	B	—	ABB	—	BDD	ABB	ABB	BCC	ABB	
251) 139446	F	76	—	ABC	B	—	ABB	—	BDD	AAB	AAB	BCC	B	
252) 139447	F	76	—	ABC	B	—	AAB	—	BDD	AAB	ABB	BCC	B	
253) 139449	F	76	—	ABC	B	—	—	—	BDD	AAB	AAB	BCC	B	
254) 139450	F	76	—	ABC	B	—	AAB	—	BDD	ABB	AAB	BCC	B	
255) 139451	F	76	—	ABB	B	—	—	—	BDD	ABB	AAB	BCC	B	
256) 140491	F	76	A	ABB	—	—	ABB	—	BDD	AAB	AAB	BCC	ABB	
Specimen	Sex	Site	Locus and gel number											
			Mdh-1 1	Mdh-2 2	Mc-1 1	Mpi 2	Pgi 2	Pgm-1 2	Pgm-2 2	Sod-1 1	Blood ( $\mu\text{m}^2$ )			
1) 129277	F	4	BBD	B	—	AAB	AAC	B	B	BDD	1135			
2) 122136	F	5	BBD	B	AAB	—	—	B	B	BDD	897			
3) 122137	F	5	BBD	B	AAB	—	—	B	B	BDD	897			
4) 122138	F	5	BBD	BBC	AAB	—	—	B	B	BDD	855			
5) 122139	F	5	BBD	B	A	—	—	B	B	BDD	895			
6) 122140	F	5	BBD	B	A	—	—	B	B	BDD	951			
7) 122141	F	5	BBD	B	A	—	—	B	B	BDD	980			
8) 122148	F	5	BBD	B	A	—	—	B	B	BDD	1085			
9) 122149	F	5	BBD	B	A	—	—	B	B	BDD	957			
10) 122150	F	5	BBD	B	A	—	—	B	B	BDD	1006			
11) 122151	F	5	BBD	B	A	—	—	B	B	BDD	988			
12) 122152	F	5	BBD	B	AAB	—	—	B	B	BDD	999			
13) 122153	F	5	BBD	B	A	—	—	B	B	BDD	886			
14) 122154	F	5	BBD	B	A	—	—	B	B	BDD	934			
15) 122155	F	5	BBD	B	A	—	—	B	B	BDD	1070			
16) 122145	F	5	BBD	B	AAB	—	—	B	B	BDD	910			
17) 122146	F	5	BBD	B	AAB	—	—	B	B	BBC	847			
18) 122142	F	5	BBD	B	AAB	—	—	B	B	BDD	962			
19) 122143	F	5	BBD	B	AAB	—	—	B	B	BDD	871			
20) 122147	F	5	BBD	B	AAB	—	—	B	B	BDD	971			
21) 122144	F	5	BBD	B	AAB	—	—	B	B	BDD	972			
22) 122120	F	6	BBD	B	AAB	—	AAC	B	B	BDD	976			
23) 122133	F	6	BBD	ABB	A	—	AAC	BCC	B	BDD	?			
24) 122126	F	6	BBD	ABB	AAB	—	AAC	BBC	B	BDD	993			
25) 122127	F	6	BBD	B	AAB	—	AAC	BCC	B	BDD	936			
26) 122125	F	6	BBD	B	AAB	—	AAC	—	B	BDD	908			
27) 122124	F	6	BBD	BBC	A	—	ACC	BBC	B	BDD	1027			
28) 122130	F	6	BBD	BBC	A	—	AAC	BBC	B	BDD	944			
29) 122128	F	6	BBD	B	A	—	AAC	BCC	B	BDD	871			
30) 122129	F	6	BBD	B	A	—	AAC	BBC	B	BDD	1053			
31) 122131	F	6	BBD	BBC	AAB	—	AAC	BBC	B	BDD	982			
32) 122121	F	6	BBD	B	AAB	—	AAC	B	B	BDD	987			
33) 122122	F	6	BBD	B	AAB	—	AAC	B	B	BDD	959			
34) 122123	F	6	BBD	B	A	—	AAC	BBC	B	BDD	880			
35) 122132	F	6	BBD	B	AAB	—	AAC	BBC	B	BDD	1196			
36) 122087	F	8	BBD	B	—	—	—	—	B	BDD	966			
37) 139537	F	9	BBD	B	—	AAB	AAC	BBC	B	BDD	1401			
38) 122088	F	10	BBD	B	AAB	—	—	BCC	B	BDD	1041			
39) 122089	F	10	BBD	B	AAB	—	—	BCC	B	BDD	909			
40) 131560	F	11	BBD	B	ABB	AAB	AAC	BBC	B	BDD	1220			
41) 131561	F	11	BBD	B	ABB	AAC	AAC	BBC	B	BDD	1016			
42) 131562	F	11	BBD	B	ABB	AAB	AAC	B	B	BDD	1082			
43) 131563	F	11	BBD	B	ABB	AAC	AAC	BCC	B	BDD	1022			
44) 131564	F	11	BBD	B	ABB	AAB	AAC	BBC	B	BDD	1116			
45) 131565	F	11	BBD	B	ABB	AAB	AAC	BBC	B	BDD	1094			

APPENDIX B-5  
(Continued)

Specimen	Sex	Size	Locus and gel number								
			Mdh-1 1	Mdh-2 2	Me-1 1	Mpi 2	Pgi 2	Pgm-1 2	Pgm-2 2	Sod-1 1	Blood ( $\mu\text{m}^2$ )
46) 131566	F	11	BBD	B	ABB	AAB	AAC	BBC	B	BDD	1163
47) 131567	F	11	BBD	B	ABB	AAB	AAC	B	B	BDD	1099
48) 131568	F	11	BBD	B	A	ABB	AAC	BBC	B	BDD	1112
49) 122101	F	12	BBD	B	—	—	—	B	B	BDD	927
50) 122102	F	12	BBD	B	—	—	—	B	B	BDD	1168
51) 122103	F	12	BBD	B	—	—	—	B	B	BDD	995
52) 122111	F	12	BBD	B	A	—	—	BBC	B	BDD	937
53) 122112	F	12	BBD	B	AAB	—	—	B	B	BDD	1106
54) 122115	F	12	BBD	B	AAB	—	—	B	B	BDD	968
55) 122116	F	12	BBD	B	AAB	—	—	B	B	BDD	1028
56) 122106	F	12	BBD	B	A	—	—	B	B	BDD	1178
57) 122105	F	12	BBD	B	A	—	—	B	B	BDD	1113
58) 122107	F	12	BBD	B	A	—	—	B	B	BDD	1097
59) 122108	F	12	BBD	B	AAB	—	—	B	B	BDD	1008
60) 122109	F	12	BBD	B	AAB	—	—	B	B	BDD	915
61) 122100	F	12	BBD	B	A	—	—	BBC	B	BDD	957
62) 122099	F	12	BBD	B	—	—	—	B	B	BDD	954
63) 122098	F	12	BBD	B	—	—	—	B	B	BDD	951
64) 122097	F	12	BBD	B	A	—	—	BBC	B	BDD	831
65) 131584	F	13	BBD	B	ABB	AAB	AAB	B	B	BDD	?
66) 131585	F	13	BBD	B	ABB	AAC	AAB	B	B	BDD	1137
67) 131586	F	13	BBD	B	ABB	AAC	AAB	BBC	B	BDD	1038
68) 131587	F	13	BBD	B	ABB	AAB	ABC	BBC	B	BDD	1166
69) 131588	F	13	BBD	B	ABB	AAC	AAB	B	B	BDD	1198
70) 131589	F	13	BBD	B	ABB	AAC	ABC	B	B	BDD	1141
71) 131590	F	13	BBD	B	ABB	ABB	ABB	B	B	BDD	1118
72) 131594	F	14	BBD	B	ABB	AAB	—	BBC	B	BDD	1082
73) 131595	F	14	BBD	B	ABB	AAB	AAB	B	B	BDD	951
74) 131596	F	14	BBD	B	ABB	ABB	AAC	B	B	BDD	1039
75) 131597	F	14	BBD	B	ABB	AAB	AAC	B	B	BDD	1216
76) 131598	F	14	BBD	B	ABB	AAB	AAB	B	B	BDD	?
77) 131599	F	15	BBD	B	ABB	AAB	ABC	B	B	BDD	1155
78) 122090	F	19	BBD	B	A	—	—	—	B	BDD	984
79) 122091	F	19	BBD	B	A	—	—	—	B	BDD	878
80) 122092	F	19	BBD	B	AAB	—	—	—	B	BDD	1020
81) 122093	F	19	BBD	B	AAB	—	—	—	B	BDD	992
82) 122094	F	19	BBD	B	AAB	—	—	—	B	BDD	1035
83) 122095	F	19	BBD	B	AAB	—	—	—	B	BDD	982
84) 122096	F	19	BBD	B	AAB	—	—	—	B	BDD	913
85) 129304	F	20	BBD	B	A	—	—	BBC	B	BDD	1019
86) 129305	F	20	BBD	B	AAB	—	—	BBC	B	BDD	932
87) 129306	F	20	BBD	B	AAB	—	—	BCC	B	BDD	1132
88) 129307	F	20	BBD	B	AAB	—	—	BCC	B	BDD	1147
89) 129308	F	20	BBD	B	AAB	—	—	BBC	B	BDD	894
90) 129309	F	20	BBD	B	AAB	—	—	BBC	B	BDD	1035
91) 129310	F	20	BBD	B	AAB	—	—	BBC	B	BDD	938
92) 134462	F	20	BBD	B	AAB	—	—	BBC	B	BDD	911
93) 135601	F	21	BBD	B	—	AAB	AAC	BBC	B	BDD	894
94) 129299	F	22	BBD	B	—	AAC	AAB	BCC	B	BDD	971
95) 129300	F	22	BBD	B	ABB	AAC	—	BCC	B	BDD	947
96) 129296	F	22	BBD	B	—	AAC	AAB	B	B	BDD	982
97) 129297	F	22	BBD	B	A	AAC	AAB	B	B	BDD	1036
98) 129298	F	22	BBD	B	—	AAC	AAB	B	B	BDD	964
99) 129292	F	22	BBD	B	—	AAC	—	BBC	B	BDD	874
100) 129314	F	22	BBD	B	—	ACC	AAC	—	B	BDD	913
101) 121991	F	23	BBD	B	—	—	—	—	B	BDD	1052
102) 114070	F	25	BBD	B	—	—	—	—	B	BDD	1028

APPENDIX B-5  
(Continued)

Specimen	Sex	Size	Locus and gel number								Blood ( $\mu\text{m}^2$ )	
			Mdh-1 1	Mdh-2 2	Me-1 1	Mpi 2	Pgi 2	Pgm-1 2	Pgm-2 2	Sod-1 1		
103)	114059	F	27	BBD	B	—	—	AAC	B	B	BDD	989
104)	114060	F	27	BBD	B	—	—	AAC	B	B	BDD	852
105)	114061	F	27	BBD	B	—	—	AAC	B	B	BDD	914
106)	114062	F	27	BBD	B	—	—	AAC	B	B	BDD	955
107)	114063	F	27	BBD	B	—	—	AAC	B	B	BDD	915
108)	114064	F	27	BBD	B	—	—	AAC	B	B	BDD	997
109)	121968	F	27	B	B	—	—	AAC	B	B	BDD	900
110)	121969	F	27	BBD	BBC	—	—	AAC	B	B	BDD	803
111)	121970	F	27	BBD	BBC	—	—	AAC	B	B	BDD	907
112)	129412	F	27	BBD	B	—	—	AAC	B	B	BDD	886
113)	114065	F	28	BBD	B	—	—	AAC	B	B	BDD	963
114)	114080	F	29	BBD	B	A	—	—	B	B	BDD	?
115)	129325	F	30	BBD	B	ABB	—	—	B	B	BDD	971
116)	129326	F	30	BBD	B	ABB	—	—	B	B	BDD	976
117)	129322	F	30	BBD	B	ABB	—	—	B	B	BDD	?
118)	129323	F	30	BBD	BBC	ABB	—	—	B	B	BDD	904
119)	129324	F	30	BBD	B	ABB	—	—	B	B	BDD	979
120)	129327	F	30	BBD	B	ABB	—	—	B	B	BDD	944
121)	129315	F	31	BBD	B	—	AAC	AAC	B	B	BDD	1047
122)	134463	F	31	BBD	B	AAB	—	AAC	B	B	BDD	871
123)	134464	F	31	BBD	BBC	ABB	—	ABC	B	B	BDD	?
124)	134484	F	31	BBD	B	ABB	—	ABC	BBC	B	BDD	1286
125)	131620	F	32	BBD	B	AAB	AAC	AAB	BBC	B	BDD	989
126)	131621	F	32	BBD	B	—	AAB	AAC	B	B	BDD	1331
127)	131625	F	32	BBD	B	AAB	AAB	AAB	BBC	B	BDD	989
128)	131626	F	32	BBD	B	AAB	AAB	AAB	B	B	BDD	1126
129)	131627	F	32	BBD	B	—	AAB	AAC	B	B	—	?
130)	131628	F	32	BBD	B	ABB	AAB	AAB	B	B	BDD	1165
131)	131622	F	32	BBD	B	ABB	AAB	AAC	B	B	BDD	1028
132)	131623	F	32	BBD	B	ABB	AAB	ABC	B	B	BDD	?
133)	131624	F	32	BBD	B	A	AAB	AAC	B	B	BDD	1101
134)	122009	F	33	BBD	B	ABB	AAC	—	B	B	BDD	?
135)	114066	F	34	BBD	B	ABB	AAC	—	—	B	BDD	1047
136)	129419	F	34	BBD	BBC	ABB	AAC	—	—	B	BDD	879
137)	129420	F	34	BBD	B	ABB	AAC	—	—	B	BDD	907
138)	134482	F	37	BBD	B	A	—	ABC	B	B	BDD	985
139)	135602	F	39	BBD	B	ABB	AAC	AAC	B	B	BDD	989
140)	129425	F	41	BBD	B	ABB	AAC	—	B	B	BDD	—
141)	129426	F	41	BBD	B	ABB	AAC	—	B	B	BDD	—
142)	129427	F	41	BBD	B	—	AAC	AAC	AAB	B	BDD	884
143)	129421	F	41	BBD	B	ABB	AAC	—	—	B	BDD	891
144)	129422	F	41	BBD	B	A	AAC	—	B	B	BDD	967
145)	129423	F	41	BBD	B	A	AAC	—	B	B	BDD	1156
146)	129424	F	41	BBD	B	AAB	AAC	AAC	B	B	BDD	803
147)	135608	F	43	BBD	B	—	AAB	AAC	BBC	B	BDD	1063
148)	135609	F	43	BBD	B	—	AAB	ABC	B	B	BDD	1065
149)	135610	F	43	BBD	B	A	—	AAC	BBC	B	BDD	1127
150)	135611	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1367
151)	135612	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1060
152)	135613	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1164
153)	135614	F	43	BBD	B	ABB	—	ABC	BBC	B	BDD	1007
154)	135615	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1144
155)	135616	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1142
156)	135617	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1092
157)	135618	F	43	BBD	B	AB	—	AAC	BBC	B	BDD	1113
158)	122004	F	44	BBD	B	ABB	—	—	B	B	BDD	746
159)	122002	F	44	BBD	B	ABB	—	—	B	B	BDD	988

**APPENDIX B-5**  
*(Continued)*

Specimen	Sex	Site	Locus and gel number									Blood ( $\mu\text{m}^2$ )
			Mdh-1	Mdh-2	Me-1	Mpi	Pgi	Pgm-1	Pgm-2	Sod-1	1	
1	2	1	2	2	2	2	2	2	2	1		
160)	122003	F	44	BBD	B	ABB	—	—	—	B	BDD	778
161)	122001	F	44	—	—	—	—	—	—	—	—	764
162)	122005	F	44	BBD	B	ABB	—	—	—	B	BDD	794
163)	122006	F	44	BBD	B	A	—	—	—	B	BDD	896
164)	122007	F	44	BBD	B	ABB	—	—	—	B	BDD	1068
165)	122008	F	44	BBD	B	ABB	—	—	—	B	BDD	1062
166)	108268	F	46	BBD	B	A	—	—	B	B	BDD	849
167)	108269	F	46	BBD	B	A	—	—	B	B	BDD	1001
168)	108270	F	46	BBD	B	—	—	—	B	B	BDD	909
169)	108271	F	46	BBD	B	—	—	—	B	B	BDD	875
170)	108272	F	46	BBD	B	—	—	—	B	B	BDD	944
171)	108273	F	46	BBD	B	—	—	—	B	B	BDD	916
172)	108276	J	46	BBD	B	—	—	—	B	B	BDD	903
173)	108025	F	46	—	B	A	—	—	B	B	BDD	?
174)	114068	F	46	BBD	B	—	—	—	B	B	BDD	808
175)	114067	F	46	BBD	B	—	—	—	B	B	BDD	946
176)	114071	F	46	—	B	—	—	—	B	B	BDD	?
177)	114069	F	46	BBD	B	—	—	—	B	B	BDD	830
178)	124702	F	46	BBD	B	—	—	—	B	B	BDD	?
179)	131663	F	46	BBD	B	ABB	AAB	AAC	B	B	BDD	1058
180)	131664	F	46	BBD	B	ABB	AAB	AAC	B	B	BDD	714
181)	131665	F	46	BBD	B	ABB	AAB	ABC	BBC	B	BDD	1600
182)	134494	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1161
183)	134495	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1050
184)	134496	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1036
185)	134497	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1114
186)	134499	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1097
187)	134500	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1150
188)	134501	F	46	BBD	B	ABB	—	AAC	B	B	BDD	974
189)	134502	F	46	BBD	B	ABB	—	AAC	B	B	BDD	1110
190)	139411	F	46	BBD	B	ABB	AAB	AAC	B	B	BDD	776
191)	139412	F	46	BBD	B	ABB	AAB	AAC	B	B	BDD	966
192)	139386	F	47	BBD	B	—	AAB	—	B	B	BDD	942
193)	139387	F	51	BBD	B	ABB	—	AAC	—	B	BDD	—
194)	139388	F	51	BBD	BBC	ABB	—	—	B	B	BDD	1080
195)	139389	F	51	BBD	B	ABB	—	—	B	B	BDD	1039
196)	139390	F	51	BBD	B	ABB	—	AAC	B	B	BDD	980
197)	139391	F	51	BBD	B	B	—	—	B	B	BDD	1202
198)	139392	F	51	—	B	—	—	—	—	B	—	1109
199)	139393	F	51	BBD	B	ABB	—	—	B	B	BDD	1121
200)	139394	F	51	BBD	B	ABB	—	—	BBC	B	BDD	1041
201)	139395	F	51	BBD	B	ABB	—	A	BBC	B	BDD	1052
202)	139396	F	51	BBD	B	ABB	—	—	B	B	BDD	1034
203)	139397	F	51	BBD	B	ABB	—	—	B	B	BDD	1047
204)	139398	F	51	BBD	B	ABB	—	AAC	B	B	BDD	947
205)	139399	F	51	BBD	B	ABB	—	AAC	B	B	BDD	1001
206)	139400	F	51	BBD	B	ABB	—	—	B	B	BDD	1000
207)	139401	F	51	BBD	B	ABB	—	AAC	B	B	BDD	1078
208)	139402	F	51	BBD	B	ABB	—	AAC	C	B	BDD	1019
209)	139403	F	51	BBD	B	ABB	—	ABC	B	B	BDD	1033
210)	139404	F	51	BBD	B	AAB	—	—	B	B	BDD	933
211)	139405	F	51	BBD	B	ABB	—	A	B	B	BDD	1097
212)	139406	F	51	BBD	B	ABB	—	AAC	B	B	BDD	984
213)	139407	F	51	BBD	B	ABB	—	ABC	BCC	B	BDD	999
214)	139408	F	51	BBD	B	ABB	—	—	B	B	BDD	1106
215)	139409	F	51	BBD	B	ABB	—	—	BBC	B	BDD	1029
216)	129335	F	52	BBD	BBC	—	—	—	B	B	BDD	—

APPENDIX B-5  
(Continued)

Specimen	Sex	Site	Locus and gel number									
			Mdh-1 1	Mdh-2 2	Me-1 1	Mpi 2	Pgi 2	Pgm-1 2	Pgm-2 2	Sod-1 1	Blood ( $\mu\text{m}^2$ )	
217)	129336	F	52	BBB	B	ABB	—	—	BBC	B	BDD	727
218)	129337	F	52	BBB	B	ABB	—	—	B	B	BDD	836
219)	129338	F	52	BBB	—	—	—	—	B	B	BDD	863
220)	129339	F	52	BBB	B	ABB	—	—	B	B	BDD	982
221)	129340	F	52	BBB	B	ABB	—	—	BBC	B	BDD	1099
222)	131638	F	54	BBB	B	—	AAB	ABC	B	B	BDD	932
223)	131639	F	54	BBB	B	—	AAB	AAC	BBC	B	BDD	957
224)	131640	F	54	BBB	B	—	AAB	ABC	B	B	BDD	936
225)	129344	F	55	BBB	B	AAB	AAB	—	B	B	BDD	956
226)	129345	F	55	BBB	B	A	AAB	—	BCC	B	BDD	970
227)	129346	F	55	BBB	B	A	AAB	—	BCC	B	BDD	955
228)	129347	F	56	BBB	B	AB	AAB	—	B	B	BDD	1066
229)	129348	F	56	BBB	B	AAB	AAB	—	B	B	BDD	1073
230)	129349	F	56	BBB	B	AB	AAB	—	B	B	BDD	1063
231)	129350	F	56	BBB	B	AB	AAB	—	B	B	BDD	887
232)	129351	F	56	BBB	B	AB	AAB	—	B	B	BDD	958
233)	129352	F	56	BBB	B	AB	AAB	—	B	B	BDD	982
234)	129353	F	56	BBB	B	AB	AAB	—	B	B	BDD	1135
235)	131641	F	56	BBB	B	ABB	AAB	AAC	B	B	BDD	?
236)	131642	F	56	BBB	B	ABB	AAB	AAC	B	B	BDD	1150
237)	131643	F	56	BBB	B	ABB	AAB	AAC	B	B	BDD	1204
238)	131644	F	56	BBB	B	ABC	AAB	AAC	BBC	B	BDD	1030
239)	131645	F	56	BBB	B	ABB	AAB	ABC	B	B	BDD	1147
240)	131635	F	63	BBB	B	ABB	AAC	AAB	BBC	B	BDD	1014
241)	131636	F	63	BBB	B	ABB	AAC	AAB	BBC	B	BDD	1095
242)	131637	F	63	BBB	B	ABB	AAC	AAB	BCC	B	BDD	1062
243)	129454	F	72	BBB	B	?	—	—	B	B	BDD	994
244)	129452	F	76	BBB	B	A	—	—	B	B	BDD	1030
245)	129453	F	76	BBB	B	ABB	—	—	B	B	BDD	970
246)	139441	F	76	BBB	B	ABB	—	AAC	BBC	B	BDD	1052
247)	139442	F	76	BBB	B	A	—	AAC	BBC	B	BDD	1026
248)	139443	F	76	BBB	B	—	AAB	AAC	BBC	B	BDD	1063
249)	139444	F	76	BBB	B	ABB	—	AAC	BBC	B	BDD	1034
250)	139445	F	76	BBB	B	—	AAB	AAC	BBC	B	BDD	1046
251)	139446	F	76	BBB	B	ABB	—	AAC	BBC	B	BDD	1033
252)	139447	F	76	BBB	B	ABB	—	AAC	BBC	B	BDD	1015
253)	139449	F	76	BBB	B	ABB	—	AAC	BBC	B	BDD	978
254)	139450	F	76	BBB	B	AAB	—	ABC	B	B	BDD	1004
255)	139451	F	76	BBB	B	AAB	—	ABC	BBC	B	BDD	1025
256)	140491	F	76	BBB	B	ABB	AAB	AAC	BBC	B	BDD	1170

There were two monomorphic loci: 6Pgd(B), Mdh-3(B).

APPENDIX B-6  
*Ambystoma (3) laterale-jeffersonianum* Tetraploid Hybrids

Specimen	Sex	Site	Locus and gel number											
			Adh	AcPh-2	Acon-1	Ck-2	Est-1	Got-1	Got-2	Idh-1	Ldh-1	Ldh-2	Mdh-1	
2	2	2	1	1	3	1	1	1	1	1	1	1	1	
1) 129276	F	3	A	A	B	AAAB	—	BBBD	ABBB	A	BBBC	A	BDDD	
2) 129287	F	7	AAAC	AAAB	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	A	BDDD	
3) 131614	F	17	C	A	BBBC	AAAB	AAAB	BBBD	ABBB	ABBB	BCCC	AAAB	BDDD	
4) 145047	F	18	—	AABB	BBBC	ABBB	—	BBBD	ABBB	ABBB	BBBC	ABBB	BDDD	
5) 134483	F	38	A	AAAB	B	—	—	BBBD	ABBB	ABBB	BBBC	A	BDDD	
6) 122016	F	40	—	—	—	—	AAAB	BBBD	ABBB	ABBB	B	B	BDDD	
7) 122042	F	42	A	—	—	—	A	BBBD	ABBB	ABBB	B	AABB	BDDD	
8) 122086	F	62	—	AAAB	BCCC	AAAB	—	BBBD	ABBB	B	BBBC	A	BDDD	
9) 129399	F	70	ACCC	—	BBBC	AAAB	—	BBBD	ABBB	ABBB	BBBC	A	BDDD	
10) 140493	F	78	A	?	—	AAAB	—	BBBD	ABBB	ABBB	B	B	BDDD	
11) 140496	F	79	A	B	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
12) 140497	F	79	A	B	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	AABB	BDDD	
13) 140498	F	79	A	ABBB	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
14) 140499	F	79	A	B	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
15) 139432	F	80	—	A	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
16) 139433	F	80	—	A	—	AAAB	—	BBBD	ABBB	ABBB	B	B	BDDD	
17) 139434	F	80	—	A	—	—	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
18) 140505	F	81	A	AAAB	—	—	—	BBBD	ABBB	ABBB	BBBC	AAAB	BDDD	
19) 134531	F	82	AAAC	AAAB	B	ABBB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
20) 134532	F	82	A	ABBB	B	ABBB	—	BBBD	ABBB	ABBB	BBBC	B	BBDD	
21) 134533	F	82	A	ABBB	BCCC	ABBB	—	BBBD	ABBB	ABBB	BBBC	B	BBBD	
22) 134551	F	84	—	AAAB	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
23) 134541	F	86	—	ABBB	—	AAAB	—	BBBD	ABBB	ABBB	BBBC	B	BDDD	
24) 134566	F	87	A	A	BBBC	AAAB	—	BBBD	AABB	ABBB	BBBC	B	BDDD	
25) MCZ 110610	F	98	?	AAAB	BCCC	AAAB	—	BBBD	ABBB	ABBB	BBBC	A	BDDD	

Specimen	Sex	Site	Locus and gel number									Blood ( $\mu\text{m}^2$ )
			Mdh-2	Mdh-3	Me-1	Mpi	Pgi	Pgm-1	Pgm-2	Sod-1		
1	2	1	2	1	2	2	1	1	1	2		
1) 129276	F	3	BBBC	B	AABB	ABBB	—	—	B	BBBD	1129	
2) 129287	F	7	B	B	—	ABBB	A	B	B	BBBD	1016	
3) 131614	F	17	B	B	ABBB	ABBB	ACCC	B	B	BBBD	1353	
4) 145047	F	18	B	B	A	ABBB	ACCC	B	B	BBBD	1386	
5) 134483	F	38	B	B	AAAB	—	ACCC	B	B	BBBD	1386	
6) 122016	F	40	B	B	—	—	—	—	B	BBBD	1035	
7) 122042	F	42	BBBC	B	A	—	—	B	B	BBBD	1212	
8) 122086	F	62	B	ABB	—	—	ACC	B	B	BBBD	1038	
9) 129399	F	70	B	B	AAAB	ABBB	—	B	B	BBBD	1138	
10) 140493	F	78	B	B	—	ABBB	—	BBBC	B	BBBD	1157	
11) 140496	F	79	B	B	—	ABBB	—	BBBC	B	BBBD	1204	
12) 140497	F	79	B	B	—	ABBB	—	B	B	BBBD	1163	
13) 140498	F	79	B	B	—	ABBB	—	B	B	BBBD	1195	
14) 140499	F	79	B	B	—	ABBB	—	B	B	BBBD	1183	
15) 139432	F	80	B	B	AAAB	—	ACCC	B	B	BBBD	1222	
16) 139433	F	80	B	B	ABBB	—	ACCC	B	B	BBBD	1248	
17) 139434	F	80	B	B	AAAB	—	ACCC	B	B	BBBD	1210	
18) 140505	F	81	B	B	A	ABBB	AAAC	B	B	BBBD	1421	
19) 134531	F	82	B	B	—	—	ACCC	B	B	BBBD	1628	
20) 134532	F	82	B	B	AAAB	—	ACCC	B	B	BBBD	1218	
21) 134533	F	82	B	B	AAAB	—	AAAC	B	B	BBBD	1301	
22) 134551	F	84	B	B	AAAB	—	BCCC	B	B	BBBD	1429	
23) 134541	F	86	B	B	AAAB	—	BCCC	—	B	BBBD	1349	
24) 134566	F	87	B	B	AAAB	—	ACCC	BBBC	B	BBDD	1135	
25) MCZ 110610	F	98	B	B	—	ABBB	AAAC	BBBC	B	BBBD	1226	

**APPENDIX B-7**  
***Ambystoma* (2) *laterale*–(2) *jeffersonianum* Tetraploid Hybrids**

Specimen	Sex	Site	Locus and gel number											
			Adh 2	AcPh-2 2	Acon-1 2	Ck-1 1	Ck-2 1	Est-1 2	Got-1 3	Got-2 2	Idh-1 1	Ldh-1 1	Ldh-2 1	
1) 131569	F	11	A	A	C	A	AABB	—	BDDD	AAAB	AAAB	BBBC	B	
2) 121988	F	23	AAAC	BBDD	CCCC	A	—	—	AABD	AABB	AABB	BBCC	B	
3) 122015	M	40	—	—	—	A	—	AABB	BBDD	AABB	AABB	B	B	
4) UMMZ 176837	F	74	—	—	—	A	—	—	BBDD	AABB	AABB	BBCC	ABBB	
5) UMMZ 176836	F	74	—	—	—	A	—	—	BBDD	AABB	AABB	BBCC	AAAB	
6) 140492	F	78	A	AABB	—	A	AAAB	—	BBDD	AABB	AABB	BBCC	ABBB	

Specimen	Sex	Site	Locus and gel number									Blood ( $\mu\text{m}^2$ )
			Mdh-1 1	Mdh-3 2	Me-1 1	Mpi 1	Pgi 2	Pgm-1 2	Pgm-2 1	Sod-1 1		
1) 131569	F	11	BBDD	B	AABB	AAAB	AAAC	B	B	BBDD	1016	
2) 121988	F	23	BBDD	B	B	—	—	BBCC	B	BBDD	1109	
3) 122015	M	40	BBDD	AABB	—	—	—	—	B	BBDD	1015	
4) UMMZ 176837	F	74	BBDD	B	—	—	—	B	B	BBDD	1053	
5) UMMZ 176836	F	74	BBDD	B	—	—	—	B	B	BBDD	1334	
6) 140492	F	78	BBDD	B	—	B	—	B	B	BBDD	1103	

APPENDIX B-8  
*Ambystoma laterale*-(3) *jeffersonianum* Tetraploid Hybrids

Specimen	Sex	Site	Locus and gel number											
			Adh-2	AcPh-2	Acon-1-2	Ck-1-1	Ck-2-1	Est-1-2	Got-1-3	Got-2-2	Idh-1-1	Ldh-1-1	Ldh-2-1	
1)	129288	F	11	A	ABBB	BCCC	A	ABBB	ABBB	BDDD	AAAB	AAAB	BCCC	B
2)	129289	F	11	A	ABBB	BCCC	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	B
3)	122110	F	12	A	ABBB	BBCC	A	—	—	BDDD	AAAB	AABB	BGCC	B
4)	122113	F	12	AAAC	ABBB	BCCC	A	—	—	BDDD	AAAB	AABB	BCCC	B
5)	131629	F	32	—	—	—	A	AABB	—	BDDD	AABB	AAAB	BCCC	AABB
6)	129430	F	41	—	—	—	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	ABBB
7)	129431	F	41	A	A	—	A	—	—	BDDD	AAAB	AAAB	BCCC	B
8)	129428	F	41	A	—	BCCC	A	—	—	BDDD	AAAB	AAAB	BCCC	B
9)	129429	F	41	—	—	—	A	—	—	BDDD	AAAB	AAAB	BCCC	B
10)	129432	F	41	A	AAAB	BCCC	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	B
11)	135619	F	43	—	ABBB	BCCC	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	B
12)	135620	F	43	—	A	—	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	B
13)	134493	F	46	—	A	BCCC	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	B
14)	134498	F	46	—	AAAB	BCCC	A	ABBB	—	BDDD	AAAB	AAAB	BCCC	B
15)	139448	F	76	—	AABC	B	A	AAAB	—	BDDD	AAAB	AAAB	BCCC	B

Specimen	Sex	Site	Locus and gel number									Blood ( $\mu\text{m}^2$ )
			Mdh-1-1	Mdh-2-2	Me-1-1	Mpi-1	Pgi-2	Pgm-1-2	Pgm-2-1	Sod-1-1		
1)	129288	F	11	BBBD	B	A	AAAB	AAAC	BBBC	B	BDDD	1229
2)	129289	F	11	BBBD	B	AABB	AAAB	AAAC	BBBC	B	BDDD	1210
3)	122110	F	12	BBBD	B	AABB	—	—	B	B	BDDD	1226
4)	122113	F	12	BBBD	B	ABBB	—	—	B	B	BDDD	1280
5)	131629	F	32	BBBD	B	—	AAAB	AABB	BBCC	B	?	1402
6)	129430	F	41	BBBD	B	—	AAAB	—	B	B	BDDD	1113
7)	129431	F	41	BBBD	B	—	AAAB	—	B	B	BDDD	1317
8)	129428	F	41	BBBD	B	A	AAAB	—	BBBC	B	BDDD	1199
9)	129429	F	41	BBBD	B	ABBB	AAAB	—	—	B	BDDD	982
10)	129432	F	41	BBBD	B	ABBB	AAAB	AAAC	BCCC	B	BDDD	1205
11)	135619	F	43	BBBD	B	—	AAAB	AAAC	BBBC	B	BDDD	1614
12)	135620	F	43	BBBD	B	AABB	—	AAAC	B	B	BDDD	1294
13)	134493	F	46	BBBD	B	ABBB	—	AABC	BBBC	B	BDDD	1414
14)	134498	F	46	BBBD	B	ABBB	—	AAAC	B	B	BDDD	1381
15)	139448	F	76	BBBD	B	A	—	AABC	BBBC	B	BDDD	1154



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